Climatic effects on population dynamics and hybridization of a rare grasshopper species

Dem Fachbereich VI (Raum- und Umweltwissenschaften) der Universität Trier zur
Erlangung des akademischen Grades Doktor der Naturwissenschaften (Dr. rer. nat.)
genehmigte Dissertation

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Datum der wissenschaftlichen Aussprache:
16.07.2015

Trier, 2015

> *“An understanding of the natural world and what’s in it*
>
> *is a source of not only a great curiosity but great fulfillment.”*
>
> \- David Attenborough -

Ch. montanus ♀

In loving memory to my parents

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Acknowledgements

There are many people who contributed to the realization of this thesis and I am deeply grateful to all of them.

First of all I want to thank my first supervisor PD Dr. Axel Hochkirch. Although I was an “outsider” from the University of Düsseldorf with focus on bee conditioning, he put trust in me and my work and introduced me in the fascinating world of grasshoppers and hybrids. I am grateful for his help and ideas planning my dissertation project as well as for discussion and critical reading of my manuscripts. Furthermore, he gave me the opportunity to become acquainted with various laboratory and statistical methods. Without his encouragement the realization of my dissertation would not have been possible in the way it is.

Likewise I want to thank Prof. Dr. Alexander Proelß, who agreed to be the second supervisor of my thesis. I am much obliged to him for inviting me to have a look inside a for me completely foreign science and for giving me the opportunity to broaden my mind. I am also grateful to him for his encouragement, input and critical reading, which enabled me to write a legal assessment on hybrids.

Furthermore, I am grateful to Prof. Dr. Günter Köhler, who agreed to be the third supervisor of my thesis.

I further want to extend my gratitude to Dr. Ortwin Elle for his untiring efforts to help me with complicated GIS questions until the perfect answer was found.

I am grateful to the German Research Foundation (DFG) and the “Forschungsinitiative Rheinland-Pfalz” for financial support and for giving me the opportunity to visit several conferences. Likewise I am grateful to the “Struktur- and Genehmigungsdirektion Nord” for the permission to collect samples and the access to nature reserves.

For help and support in the field as well as for a great time I want to thank Nicole Kranz, Marco Kranz and particularly Jasmin Weinberger, who supported me over three field seasons. Furthermore, I want to thank Elena Dreher for her dedication in the laboratory experiments as well as Elena Kaut for support in the lab. I would also like to express my gratitude to all technical assistants of our lab for always supporting me. Special thanks go to Karin Fischer and Sabine Naber, who not only helped me with genotyping a high number of samples, but also supported me and my dissertation in their free time.

For proofreading parts of this thesis and the willingness to discuss I am thankful to Subashini Arichdran, Aleke Stoefen-O’Brien and Marcus Fingerle.

I am very grateful to all members of the Department of Biogeography and the Graduate School for making the dissertation time something special for me. Special thanks go to Ute Herrmann for her support in all administrative questions.

Furthermore, special thanks go to Maren Hochkirch and kids as well as to Sarah and Roswitha Wirtz for their great private support.

I sincerely thank all my friends and my family for encouragement and distraction, particularly during the rough time at the beginning of my doctorate. Special thanks go to my office mate Daniela Matenaar for her amity and support during the whole time, as well as for her willingness to discuss and for great conference journeys. Furthermore, I am deeply grateful to Marcus Fingerle for a great and special time, for bearing my ups and downs, as well as for the strong and affectionate backing.

Although they could not join me on this journey, I will always be grateful to my parents, who supported me with their love. Their fascination for our impressing nature has also aroused my curiosity and brought me on this path.

Thank you!

Preliminary remarks

This PhD thesis was carried out at the Department of Biogeography, Faculty of Regional and Environmental Sciences, Trier University. Financial support was provided through the interdisciplinary graduate school ‘Cooperation of Science and Jurisprudence in Improving Development and Use of Standards for Environmental Protection – Strategies for Risk Assessment and Management’ funded by the German Research Foundation (DFG, GRK 1319) and the “Forschungsinitiative Rheinland-Pfalz”. This thesis compromises four chapters, which are either already published or submitted manuscripts (unpublished), except for the legal part (Chapter IV). The citation style is adapted to the respective disciplines. Most of the results have been presented at scientific congresses. Conference and journal contributions are listed below. Manuscripts that emerged from the interdisciplinary cooperation within the graduate school as well as publications or congress contributions with another scientific background (cooperation with the University of Düsseldorf) are listed in the *Curriculum vitae*. These publications are not included in this thesis.

Table Percentage of equity ratio on the four chapters of this thesis.

Chapter	data collection & processing	data analysis	Writing of the manuscript
I	50	70	80
II	60	80	90
III	80	90	90
IV	-	100	100

Full papers (peer reviewed)

1. **Rohde K.**, Dreher E., Hochkirch A. (2015): Sex-specific phenotypic plasticity in response to the trade-off between developmental time and body size supports the dimorphic niche hypothesis. **Biological Journal of the Linnean Society**, 2015, 115, 48-57. DOI: 10.1111/bij.12460.
2. **Rohde K**., Hau Y., Weyer J., Hochkirch A. (2015): Wide prevalence of hybridization in a rare wetland grasshopper increases genetic diversity in its widespread congener. **BMC Evolutionary Biology**, 2015, in press.

Submitted (unpublished) manuscripts:

1. **Rohde K.,** Hau Y., Kranz N., Weinberger J., Elle O., Hochkirch A. (submitted Feb. 2015) Extreme climatic events cause rapid population declines of a rare wetland species and increase hybridization risk with a habitat generalist. Submitted.

Contributions to congresses (Talks)

2014 **Rohde K.,** Weyer J., Hau Y., Lemke I., Hochkirch A.: "Hybridization - an extinction risk"; Xth European Congress of Entomology (ECE), 3rd - 8th August 2014, York (GB).

2014 **Rohde K.,** Weyer J., Hau Y., Lemke I., Hochkirch A.: "Hybridisierung – ein Risiko für gefährdete Arten?"; 13. Jahrestagung der Deutschen Gesellschaft für Orthopterologie (DGfO), 28th - 30th March 2014, Salzburg (AUT).

2013 **Rohde K.**, Weyer J., Hau Y., Lemke I., Hochkirch A.: "Hybridization – an extinction risk." 11th International Congress of Orthopterology, 11th - 15th August 2013, Kunming (CN).

General Introduction

Climate change

Climate change is one of the most controversially discussed phenomena affecting human well-being, biodiversity and ecosystems. Despite the natural variability of climate due to influences such as volcanic eruptions or solar irradiance, it is generally agreed that recent climate change is mainly induced by human activities (IPCC, 2013b). The impact of human induced carbon dioxide (CO_2) emission on climate was first described in 1896 by the chemist and physicist Svante Arrhenius (Arrhenius, 1896). Although he predicted a climate warming with ongoing CO_2 emission in the future, the consequences for the human kind and the environment were hard to predict. After decades of weather records the changing climate meanwhile becomes apparent. The anthropogenic CO_2 emission increased about 40% since the pre-industrial times, whereby from 1880 to 2012 the globally averaged combined land and ocean surface temperature increased by 0.85°C (IPCC, 2013b; Fig. 1). Consistent with climate warming, multiple environmental changes have been documented, such as rising sea levels (Cazenave, Nerem, 2004), shrinking sea ice extent in the arctic summer (Serreze *et al.*, 2007), and decreasing spring snow cover in the northern hemisphere (IPCC, 2013a; Paul *et al.*, 2004). Furthermore, the weather records provide evidence for a global accumulation of extreme climatic events such as extreme temperatures (Fischer, Schär, 2010), high precipitation (Coumou, Rahmstorf, 2012), droughts (Dai, 2011), and tropical cyclones (Webster *et al.*, 2005) which will further increase with ongoing climate warming (IPCC, 2013a). In Europe, there has been an increasing trend in the frequency of heat waves (Perkins *et al.*, 2012) and extreme precipitation (Westra *et al.*, 2013) since the middle of the 20th century regarding that its extent may vary regional and seasonal (Collins *et al.*, 2013).

Even though the general effects of greenhouse gases and aerosols on climate are well known, they will unlikely to be stopped due to enduring industrialization and ongoing extraction of fossil fuels. Even if CO_2 emission was stopped, greenhouse gases would persist and climate change would continue (IPCC, 2013b). Various climate models exist and provide information on potential future scenarios of climate change depending on parameters such as concentrations of greenhouse gases, socio-economic factors and radiative forcing. In 2007, the concept of radiative forcing (RF) was presented by the Intergovernmental Panel on Climate Change (IPCC) and should improve the assessment of anthropogenic and natural drivers of climate change (Forster, 2007). RF describes the change in the radiative energy budget of the Earth's climatic system due to external factors (e.g. greenhouse gases, aerosols, sun irradiance, land-use etc.) at the tropopause expressed as watts per square meter of surface (W/m², Forster, 2007). Usually, the pre-industrial year 1850 is chosen as baseline for computing the amount of RF. RF can be linked through a linear relationship to the global mean equilibrium temperature change at the surface (Forster, 2007), i.e. a positive radiative forcing indicates global warming, whereby a negative radiative forcing represents a decrease in temperature.

The IPCC (2013a) presented four Representative Concentration Pathways Scenarios (RCP Scenarios), which are based up on multiple scenarios from the scientific literature. Predefined concentrations of greenhouse gases provide the basis for these RCP Scenarios, which refer to the carbon cycle model. As CO_2 is believed to be the largest contributor to anthropogenic RF, it is chosen as reference gas (i. e. Carbon dioxide equivalent, CO2-eq, expressed as units of parts per million by volume (ppmv); Gohar, Shine, 2007). The RF calculated in the RCP8.5 scenario for example predicts an increase of 8.5 W/m² in 2100 relative to the pre-industrial time in 1850, if the concentration of greenhouse gases will reach 1370 ppmv CO_2-eq (IPCC, 2013b; Fig. 1). Based on these climate models, the IPCC (2013a) forecasts an increase of the global mean surface temperature from the pre-industrial time (1850-1900) to the end of the 21st century exceeding 1.5°C (low emission scenario, RCP2.6) up to a maximum of 4.5°C (high emission, scenario, RCP8.5; Fig. 1). Furthermore, these long-term projections predict higher surface air temperatures in northern polar regions than in the tropics and the southern polar regions (Collins *et al.*, 2013). Additionally, long-term scenarios suggest, under the most extreme emission scenario, an increase of precipitation up to 50%, particularly in parts of the tropics and polar latitudes, whereas precipitation may decrease about 30% or more in large parts of the subtropics. Furthermore, an increasing frequency of extreme climatic events such as heat waves, extreme cold winters and extreme precipitation is predicted (Ballester *et al.*, 2010; Emori, Brown, 2005; IPCC, 2013a).

Regional climate scenarios for Central Europe (Ciscar *et al.*, 2009) and Germany (Jacob *et al.*, 2008) predict an increase in mean surface temperature between 2.5 and 3.5°C, an accumulation of heat days (temperature > 30°C) and tropical nights (temperature > 20°C) as well as increasing extreme precipitation events until the end of the 21st (Beniston *et al.*, 2007; Jacob *et al.*, 2008). The frequency and intensity of heat waves as well as winter precipitation will increase with high probability, whereas summer precipitation is predicted to decrease up to 25%, particularly in south and southwestern Germany (Ballester *et al.*, 2010; Beniston *et al.*, 2007; Jacob *et al.*, 2008). In the coastal

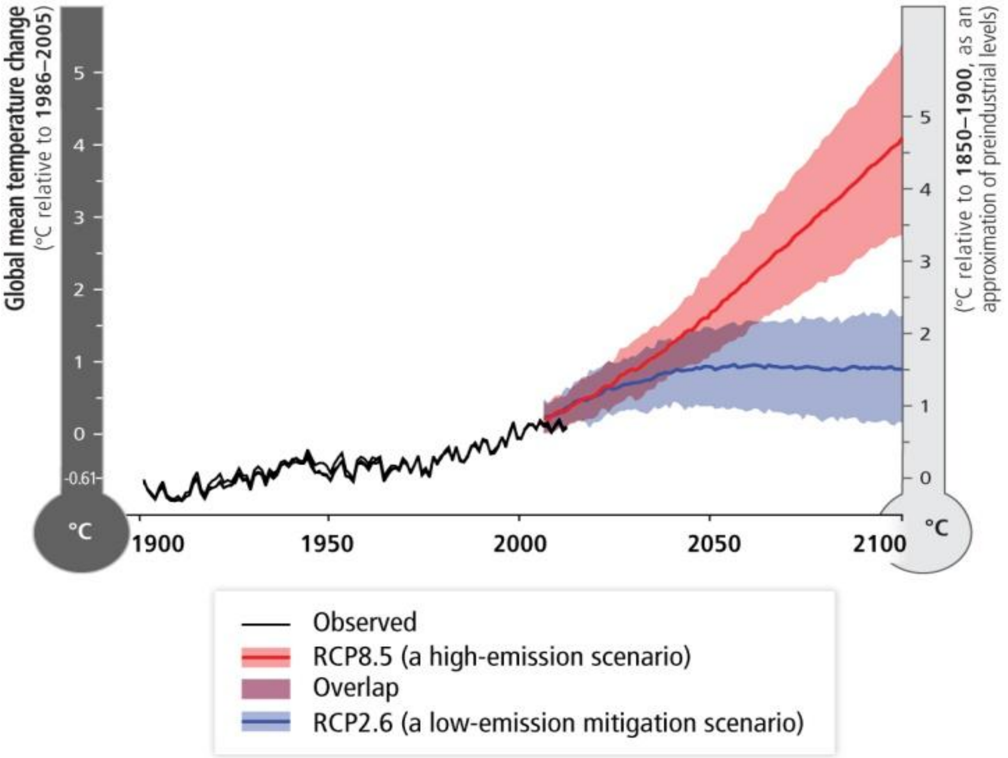

Figure 1 Observed and projected global annual surface temperature as indicated by different emission scenarios (RCP = Representative Concentration Pathways). Modified after IPCC 2014 (Technical Summary; Assessment Box SPM.1 and Fig.1).

regions of Germany, Netherlands and Denmark a higher frequency of winter storms is expected (Beniston *et al.*, 2007; Jacob *et al.*, 2008). Moreover, the projections indicate an increasing variability of climate (Jacob *et al.*, 2008).

However, it should be noted that current and future internal natural variability represents a source of uncertainty for projections of climate and extreme climatic events, particularly at smaller spatial scales (Beniston *et al.*, 2007; Collins *et al.*, 2013; Jacob *et al.*, 2008).

The impact of climate change on biodiversity and life-history traits

Besides their intrinsic value, ecosystems and biodiversity play a key role for human well-being as they provide multiple ecosystem services (e.g. food and water supply; atmospheric, climate and soil regulation; aesthetic goods). The IPCC (2014) assumed that global warming increases risks to people, economies and ecosystems substantially. Particularly rare ecosystems and cultures are already threatened by the changing climate (IPCC, 2014; Fig. 1). The direct and indirect impacts of climate change on species and ecosystems such as range shifts, phenological changes, physiological changes as well as changes in species interactions (trophic interactions, interspecific competition, hybridization) are well documented (Fig. 2; Bellard *et al.*, 2012; Cahill *et al.*, 2013; Chunco, 2014; IPCC, 2013b; Isaac, 2009; Parmesan, 2006; Parmesan *et al.*, 2000; Thomas *et al.*, 2006). Considering different dispersal abilities, physiological tolerances and life history strategies, the extent and rate of responses to climate change strongly depends on the species concerned (Foden *et al.*, 2009). Due to their short generation times and high reproductive rates, some insect groups are thought to be able to respond faster to climate change than long-lived species (Bale *et al.*, 2002; Sánchez-Guillén *et al.*, 2014). Furthermore, generalist species with higher ecological tolerances and/or higher dispersal capabilities have an advantage compared to habitat specialists. In general, three main types of responses to climate change through phenotypic plasticity and evolutionary (genetic) response have been described: range shifts, temporal shifts of life-histories traits (phenology) and physiological or behavioral shifts of life history traits (Bellard *et al.*, 2012).

Species' range shifts are among the best documented responses to climate change. It is hypothesized that range shifts in response to climate change will be more common than adaptation to warmer temperatures *in situ* (Huntley, 1991; Parmesan, 2006). As a consequence of recent global warming many species, particularly highly mobile insects such as Odonata and Lepidoptera, have already shifted their geographic ranges polewards and/or to higher elevations (Chen *et al.*, 2011; Hickling *et al.*, 2006; Parmesan, 2006; Thomas, 2010; Warren *et al.*, 2001). In extreme cases such expansions of the range boundary are accompanied by extinction processes at lower elevations and/or at the lower latitude range boundary (Parmesan, 2006; Warren *et al.*, 2001). Climate change is deemed to be one of the important drivers of biological invasions as native species may become non-native as a consequence of range expansion or show better adaptations in a newly colonized region than native species (Diez *et al.*, 2012; Hellmann *et al.*, 2008). Asynchrony in range shifts of allopatric species could lead to secondary contacts, which in turn may form novel sympatric populations (Sánchez-Guillén *et al.*, 2014). Furthermore, such asynchronies could alter dynamics and interactions of sympatric populations by increasing or decreasing their degree of sympatry (Sánchez-Guillén *et al.*, 2014; Sánchez-Guillén *et al.*, 2013). In most cases, range shifts or invasions of species have significant impacts on biodiversity. The consequences are diverse and may affect inter- and intraspecific interactions (Chunco, 2014; Parmesan, 2006; Sánchez-Guillén *et al.*, 2014; Walther *et al.*, 2002). Range-shifts may promote the displacement or even local extinction

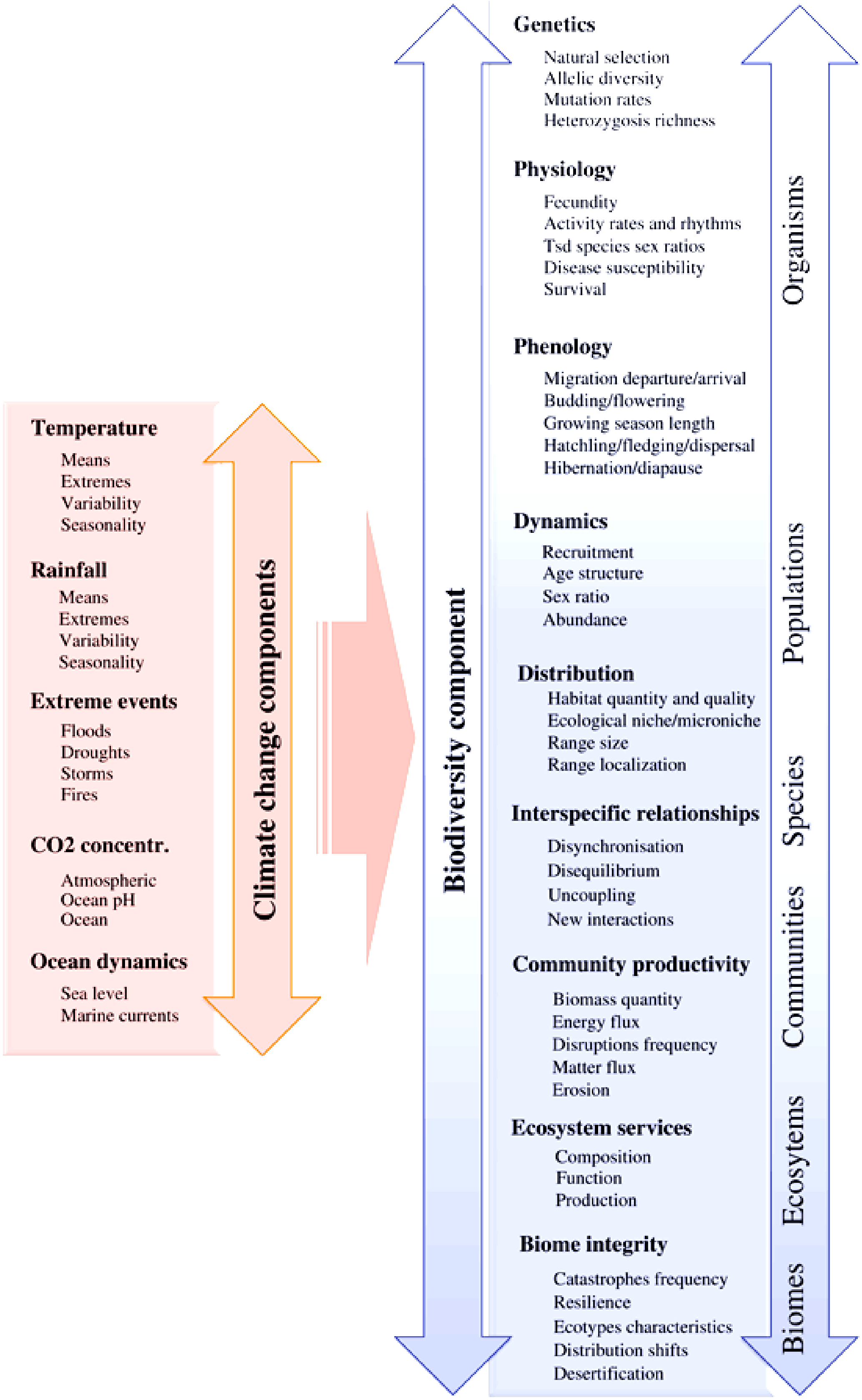

Figure 2 Summary of some of the observed and predicted aspects of climate change and some examples of likely effects on different levels of biodiversity (Modified after Bellard et al. 2012).

of native species due to increasing interspecific competition or by changing reproductive paths (e.g. hybridization; Garroway *et al.*, 2010).

The second major response to climate change are shifts of species' phenologies (Parmesan, 2006; Parmesan, Yohe, 2003; Rosenzweig *et al.*, 2007). An advanced appearance of insects, birds and amphibians has frequently been documented as well as an earlier timing of spring events with temperature increases (2-5 days per decade; Menzel *et al.*, 2006; Parmesan, 2006; Root, Hughes, 2006; Root *et al.*, 2003; Walther *et al.*, 2002). Asynchronic phenological shifts between interacting species can lead to mismatches in life history events and may have severe consequences for individual fitness and population persistence by altering trophic interactions, food availability and interspecific competition (Both *et al.*, 2009; Parmesan, 2006; Parmesan, Yohe, 2003; Root, Hughes, 2006; Visser, Both, 2005). Our knowledge on the intraspecific consequences of climate-induced phenological shifts is still scarce, but there is increasing evidence that it may have an enormous impact on the reproductive patterns of species (Forrest, Miller-Rushing, 2010). Heard et al. (2012) suggest that the variation of climate change across a species' range promotes variability of phenological responses within and among populations. Phenological isolation or connection may alter existing patterns of gene flow and affect species persistence (Heard *et al.*, 2012). Phenological isolation may facilitate the differentiation between previously connected populations and may accelerate local adaptations and even speciation. However, for small populations phenological isolation is more likely to result in inbreeding depression and in extreme cases in extinction (Heard *et al.*, 2012). As phenological isolation, increasing connectivity can have positive or negative impacts on the population involved. The admixture among populations may promote genetic diversity and adaptation as new alleles will be integrated in the population (Heard *et al.*, 2012; Schulte *et al.*, 2012), but it could also lead to outbreeding depression and genetic displacement (e.g. by hybridization; Chunco, 2014; Heard *et al.*, 2012; Rhymer, Simberloff, 1996). This applies to already genetically distinct populations of one species, as well as to populations of two sympatric species (Chadwick *et al.*, 2006; Chunco, 2014; Heard *et al.*, 2012). Chadwick et al. (2006) observed that climate change may have different effects on sympatric species, as well as on both sexes. Thus, an asynchrony of the sexes could result in a temporal separation of males and affect their reproductive success. However, studies showing such phenological sexual dimorphism induced by climate or environmental changes are still scarce.

The third common response to climate change is the shift of physiological life history traits (e.g. metabolic rates, body size, development time, reproduction) or behavior (e.g. diet, activity, thermoregulatory behavior), which enables a species to adapt to new environmental conditions *in situ* (Bellard *et al.*, 2012). Being less obvious than range shifts or phenological changes, this research field is still largely unexplored (Bellard *et al.*, 2012; Chown *et al.*, 2010; Helmuth *et al.*, 2005). Many physiological traits such as developmental time, hatching date as well as sex determination depend on climatic conditions and are thus influenced by climate change, particularly in ectotherms (Deutsch *et al.*, 2008; Réale *et al.*, 2003; Walther *et al.*, 2002). It is often not distinguished, if the physiological and behavioral adaptations to climate change are based on phenotypic plasticity or on evolutionary (genetic) responses (Bellard *et al.*, 2012; Chown *et al.*, 2010; Gienapp *et al.*, 2008; Helmuth *et al.*, 2005). Nonetheless, species with greater temperature tolerances such as habitat generalists are usually more adaptable than habitat specialists with lower temperature tolerances (Gilchrist, 2000). Moreover, foraging, mating and growth could be altered by temperature changes outside the tolerance range and may lead to fitness loss (Chown *et al.*, 2010; Gilchrist, 2000). This in turn, could alter the relative abundances of species pairs, which may affect inter- and intraspecific interactions. Physiological or behavioral adaptations to climate change are essential for species without dispersal ability in order to avoid extinction (Bellard *et al.*, 2012; Chown *et al.*, 2010; Helmuth *et al.*, 2005). It is likely that not all species will be able to keep pace with climate change (Kelly *et al.*, 2010; Malcolm *et al.*, 2006; Sandel *et al.*, 2011; Schloss *et al.*, 2012; Warren *et al.*, 2001). The ability to track climate change depends on the speed of climate change itself and on the ability of a species to respond rapidly enough in one of the aforementioned ways. Nevertheless, it must be considered that the combination of anthropogenic disturbances such as habitat destruction or deterioration, fragmentation, land use change and climate change represents a greater risk to ecosystems and global biodiversity than climate change alone (Mantyka-Pringle *et al.*, 2012; Thomas *et al.*, 2004; Travis, 2003).

Reproductive barriers, hybridization and the impact of anthropogenic disturbance

The impact of climate change on range and phenological shifts, as well as physiological and behavioral changes is of particular interest, as it may affect reproductive barriers among species (Chunco, 2014; Garroway *et al.*, 2010). These barriers may be either pre- or postzygotic mechanisms, which prevent genetic exchange between two genetically distinct species or populations and lead to reproductive isolation (Coyne, Orr, 2004). It is assumed that reproductive isolation often arises as a by-product of divergent ecological adaptation. Coyne & Orr (2004) reviewed three types of ecological isolation: pollinator isolation, habitat isolation and temporal isolation. For this thesis, mainly the latter two types are of importance. Habitat isolation is the spatial separation of species, which can be either microspatial or macrospatial. Microspatial isolation is, in general, found in sympatric species which show genetically based differences in their ecological adaptation. It restricts the species to their preferred habitats within the same geographic range. By contrast, macrospatial isolation exists, if two species show an allopatric distribution due to geographic barriers, which in turn could promote the evolution of further isolating barriers. Thus, habitat isolation prevents reproduction between species by minimizing the probability to encounter heterospecific mates. The same applies to temporal isolation, which prevents encounters of heterospecific mates by different phenologies or reproduction times. Coyne & Orr (2004) summarized that temporal isolation in animals is mainly mediated by different responses to environmental conditions based on genetic differences. Beside ecological isolation, behavioral or sexual isolation play an important role as reproductive barriers, particularly in sympatric species. Different mating signals are used to attract conspecific mates rather than heterospecifics. The evolution of such behavioral barriers may accumulate with genetic divergence or with sexual or natural selection. Another non-ecological barrier is mechanical isolation, which occurs if the reproductive organs of two species are not compatible. Furthermore, gametic isolation may occur, which can be pre- or postzygotic, i.e. it may include several traits from copulation to fertilization (cf. Coyne, Orr, 2004). It is assumed that gametic isolation is based on genetic differences in response to sexual selection. The last form of reproductive isolation is postzygotic isolation, which is based on hybrid fitness. Hybrids could have reduced viability or be sterile, which prevents genetic exchange between species. Sometimes hybrids are fertile, but not well adapted to the ecological conditions which reduce hybrid vigor (i.e. positive effects of heterosis). Furthermore, hybrids are often intermediate in behavior, which could impede mate finding as they might be unattractive for the parental species (Gottsberger, Mayer, 2007).

The strength of pre- and postzygotic reproductive barriers between species strongly depends on the environmental circumstances. Range shifts or the introduction of invasive species may reduce habitat isolation of species (Chunco, 2014; Garroway *et al.*, 2010; Rhymer, Simberloff, 1996; Sánchez-Guillén *et al.*, 2013) and phenological shifts may alter temporal isolation of sympatric species (Chunco, 2014). In addition, postzygotic barriers may be influenced, if environmental conditions suddenly favor hybrids (Chunco, 2014). Thus, the breakdown of reproductive barriers caused by environmental changes could promote hybridization (Heard *et al.*, 2012; Levin *et al.*, 1996; Rhymer, Simberloff, 1996).

Hybridization is defined as the interbreeding of two genetically distinct individuals, which belong to different populations or species (cf. also Barton, Hewitt, 1985; Seehausen *et al.*, 2014). Coyne & Orr (2004) defined that populations may represent different species, when "*(a) their genetic differences preclude them from living in the same area, or (b) they inhabit the same area but their genetic differences make them unable to produce fertile hybrids"*. Nonetheless, boundaries between species are often "fuzzy" (Agapow *et al.*, 2004) and former effective reproductive barriers can be altered by environmental changes (Hasselman *et al.*, 2014; Seehausen, 2006). Thus, speciation should be more regarded as a continuum and hybridization between species may occur at variable rates and with variable fertility (Hochkirch, 2013). Hybridization processes between two genetically distinct populations may constitute a transition period in speciation and the species status would not be definable after several species concepts (Agapow *et al.*, 2004; Coyne, Orr, 2004; Hochkirch, 2013). In order to avoid conflicts with species concepts, some definitions of hybridization ignore the taxonomic status and refer only to the genetic differences of populations (Rhymer, Simberloff, 1996).

Hybridization can be divided into two categories, natural and anthropogenic hybridization. Mallet (2005) assumed that 25% of plants and 10% of the European animals show natural hybridization. Natural hybridization constitutes an evolutionary consequence to natural range extensions or changing environmental conditions and it often leads to the formation of a stable hybrid zone zone (Allendorf *et al.*, 2013; Barton, Hewitt, 1985; Chunco, 2014). The consequences can be positive or negative and depend on the populations involved. However, hybridization can also result from anthropogenic disturbances (e.g. climate change, habitat loss, invasive species) and has therefore become an increasingly important field for conservationists (Allendorf *et al.*, 2001; Chunco, 2014; Levin *et al.*, 1996; Rhymer, Simberloff, 1996; Seehausen, 2004). It is assumed that anthropogenic disturbances contributed to increasing extinction rates due to hybridization in recent decades (Allendorf *et al.*, 2013; Coyne, Orr, 2004; Crispo *et al.*, 2011; Hasselman *et al.*, 2014; Rhymer, Simberloff, 1996; Seehausen, 2006). Regardless of the category (natural or anthropogenic), the outcomes of hybridization can differ to a significant degree. One consequence might be the extinction of species caused by genetic displacement (Rhymer, Simberloff, 1996; Seehausen *et al.*, 2008a). Even interbreeding without successful reproduction could lead to extinction by reducing the reproductive success of the interacting species (Allendorf *et al.*, 2013; Hochkirch, 2013). Furthermore, hybridization can lead to the creation of a new (hybrid) species, if hybrids show a better adaptation to environmental conditions than the parental species (Rieseberg, 1997). Hybridization can also improve the genetic diversity of genetically depleted populations, which may prevent inbreeding depression at low population size (Arnold *et al.*, 2008; Schulte *et al.*, 2012), and result in a better adaptation of the parental species to changing conditions (Baskett, Gomulkiewicz, 2011; Rieseberg *et al.*, 2003).

Among the best studied model systems for hybridization are natural hybrid zones. A hybrid zone occurs, if two genetically distinct populations (or species) come into contact, mate and produce hybrid offspring (Barton, Hewitt, 1985). In general, hybrid zones result from a secondary contact of parapatric species (Allendorf *et al.*, 2013; Barton, Hewitt, 1985). Hybrid zones are widely distributed in animal and plant taxa (reviewed Barton & Hewitt (1985) and may even occur between sympatric species, if the species are separated by their habitat requirements. Such hybrid zones within largely overlapping ranges received only little attention by evolutionary biologists and conservationists as it is often assumed that sympatric species pairs must have evolved effective reproductive barriers (Barton, Hewitt, 1989; Bettles *et al.*, 2005; Buggs, 2007; Hasselman *et al.*, 2014; Hochkirch, Lemke, 2011; Rundle, Schluter, 1998). Indeed, the differential habitat affiliation of two species may be considered such a pre-mating barrier and thus, species in sympatry do not necessary live in syntopy. However, in areas with neighboring habitats of both species even such species pairs could come into secondary contact. Therefore, reproductive barriers do not necessarily have to be complete, which in turn may lead to the formation of local hybrid zones. In most cases, hybrids of two species are less fit, which prevents the complete admixture of both parental species and enables the persistence of hybrid zones (Barton, Hewitt, 1985). Nonetheless, hybrid zones may be altered by environmental fluctuations or represent a transition stage in evolution, which may result in movements of the hybrid zone (Engler *et al.*, 2013) or even extinction of one species.

The detection of hybrids or hybrid zones is often accidentally. In general, the first indication of hybridization is the detection of intermediate morphotypes. In recent years, molecular approaches for detecting hybrids based on molecular genetic markers have been developed and improved (Anderson, Thompson, 2002; Jombart, 2008; Pritchard *et al.*, 2000). These methods replaced the less accurate methods of morphological identification of hybrids. Hybrid detectability with molecular methods depends strongly on the marker set, the threshold selected for assigning genotypes, as well as on the interpretation of the genetic data (Garroway *et al.*, 2010; Lorenzini *et al.*, 2014). Although molecular methods are efficient for identifying first generation hybrids, it is often much more difficult to distinguish second generation hybrids or backcrosses or even further generations from the parental species as their genome would be rapidly mixed up (Allendorf *et al.*, 2001). Therefore, new statistical approaches for assigning individuals to different hybrid classes have been developed (Anderson, Thompson, 2002; Jombart, 2008). Besides hybrid identification, genetic methods allow scientists to evaluate and predict possible outcomes of hybridization (e.g. genetic diversity; inbreeding or outbreeding depression).

The current extent of anthropogenic hybridization is unknown, although several examples indicate a dramatically increase in recent years (Allendorf *et al.*, 2013; Levin *et al.*, 1996; Zamundio, Harrison, 2010). It is argued that anthropogenic hybridization represents a major threat, particularly for declining species that are threatened also by other factors (Allendorf *et al.*, 2001; Rhymer, Simberloff, 1996). However, assessing and managing hybridization is an enormous challenge for conservationists taking into account that the causes and consequences of hybridization are diverse and idiosyncratic (Zamundio, Harrison, 2010). Allendorf *et al.* (2001) presented suggestions how to deal with hybrids or hybrid populations in conservation. The authors proposed six categories of hybridization depending on the degree of introgression and the context in which it emerges (Fig.3). The authors lined out that the three natural types of hybridization (Fig. 3) should not play a major role in species conservation as they represent natural evolutionary processes. By contrast, they propose that conservation management is necessary in the case of anthropogenic hybridization. However, the course of action depends on the degree of introgression. In case of hybridization without introgression (TYPE 4), the removal of hybrids and the non-native species is proposed. In case of widespread introgression (TYPE 5), the authors suggest an improved conservation of the remaining purebred population. In the case of complete admixture (TYPE 6), the authors propose a conservation of the hybrid population to ensure its survival and to preserve genetic diversity. Although this approach represents a major step forward in biodiversity conservation, it is not applicable to all situations. The authors mainly refer to anthropogenic hybridization caused by the introduction of non-native species rather than to interspecific hybridization due to climate change or habitat destruction. The decision for a removal of a native taxon is much more difficult as hybridization induced by climate change might be considered natural (cf. Chapter IV).

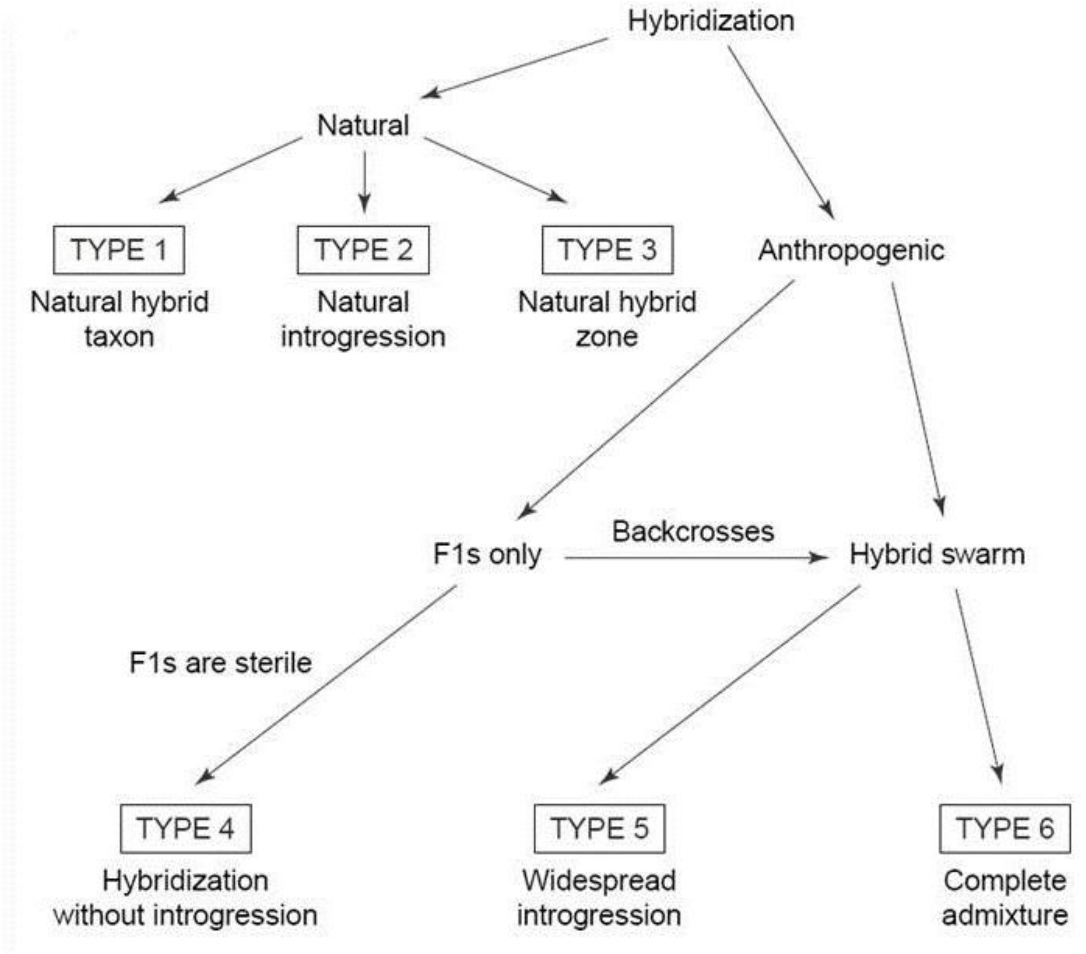

Figure 3 Framework to categorize hybridization. Each type should be viewed as a general descriptive classification used to facilitate discussion rather than a series of strict, all-encompassing divisions. Types 1-3 represent hybridization events that are a natural part of the evolutionary legacy of taxa; these taxa should be eligible for protection. Types 4-6 divide anthropogenic hybridization into three categories that have different consequences from a conservation perspective (from Allendorf *et al.* 2001).

This section underlines that although climate change is not a new phenomenon, little is known about the effects of climate change on intra- and interspecific interactions such as hybridization or its impact on existing hybrid zones (Chunco, 2014). Indeed, newly emerging climate-mediated hybrid zones have been documented (Chunco, 2014). In order to assess the impact of climate change on species interactions and to develop appropriate conservation tools, a further improvement in molecular approaches, laboratory experiments, as well as in modeling species distribution or ecological niches in relation to different climate scenarios is required (Chunco, 2014; Garroway *et al.*, 2010; Lorenzini *et al.*, 2014; Zamundio, Harrison, 2010). In addition, it is necessary to consider climate-mediated hybridization in combination with other anthropogenic disturbances, particularly in combination with habitat loss, fragmentation and the introduction of invasive and domesticated species.

The focus species: a rare wetland specialist

The grasshopper species *Chorthippus montanus* (Charpentier, 1825) represents an excellent model system for the study of climate-mediated responses in life-history traits and interspecific interactions. As a hygrophilous species it represents a suitable indicator for permanently moist habitats, such as marshes and peat bogs and is thus likely to respond strongly to climate change. Moreover, it is known to be able to hybridize with *Chorthippus parallelus* (Zetterstedt, 1821), its sibling species (Vedenina, Mugue, 2011). Both species have very similar morphology and life history. They are both flightless, but occasionally produce fully alate macropterous specimens. Furthermore, they have a strongly overlapping distribution, which makes this species pair particularly interesting for the study of hybridization in naturally sympatric populations. Both species are univoltine, which enables the investigation of several non-overlapping generations in a short period of time. This is important to assess long-term changes in population dynamics and genetic diversity.

Chorthippus montanus is distributed across Eurosiberia from Western Europe to Kamchatka (Fröhlich, 1994; Ingrisch, 1983a; Kleukers *et al.*, 1997). Its adaptation to wetlands is mainly caused by the hygrophilous eggs, which need constant soil moisture for embryonic development (Ingrisch, 1983a). *Ch. montanus* is one of the grasshopper species that emerges latest in the year in Central Europe (Detzel, 1998). Nymphs hatch in May and become adult from end of July to mid of November (Weyer *et al.*, 2012). Due to ongoing habitat fragmentation, drainage and land use changes, *Ch. montanus* populations are frequently threatened with extinction (Weyer *et al.*, 2012). As it is a brachypterous species, its dispersal capability is low and populations are often restricted to their habitats (Reinhardt *et al.*, 2005; Weyer *et al.*, 2012). These properties offer the opportunity to study the impact of climate change (in combination with other threats) on life history traits and population ecology of a highly specialized species in field studies as well as under laboratory conditions.

In the genus *Chorthippus* various hybrid zones have been documented (Bridle *et* *al.*, 2001; Gottsberger, 2007; Hewitt, 1993). Among the best studied hybrid zones is certainly the hybrid zone of the two subspecies *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* in the Pyrenees (Hewitt 1993). *Ch. parallelus* is a widespread habitat generalist which occurs in most non-arid grassland habitats (Detzel 1998). Compared to *Ch. montanus*, *Ch. parallelus* has a slightly shifted phenology (Baur *et al.*, 2006; Detzel, 1998). *Ch. parallelus* nymphs hatch usually ca. one month earlier than *Ch. montanus*. Adults are found from June to October. Both species are morphological very similar and have similar songs, which gave rise to the assumption that hybridization could occur in syntopic populations (Bauer, von Helversen, 1987; Reynolds, 1980). Laboratory experiments showed that hybrid offspring is fertile (at least to the second generation) and corroborated this assumption (Köhler, 2013). Research on syntopic populations of two sympatric, native species with different ecological amplitudes (specialist versus) could provide new insights into the processes behind interspecific hybridization, reproductive barriers and how anthropogenic disturbances may alter these interactions.

Structure of this thesis

The central goal of this thesis was to gain deeper insights in the effects of climate on life-history traits, population dynamics as well as interspecific interactions of a habitat specialist (*Ch. montanus*). Additionally, this thesis emphasizes the role of hybrids and hybridization in nature conservation and environmental law and policies.

The experiment conducted in **Chapter I** was intended to test if macropterism in *Ch. montanus* is determined by temperature or population density, as has been shown for other Orthoptera (Poniatowski, Fartmann, 2011b). This would provide some information if global warming might not only threaten this species by increasing droughts but also could trigger new dispersal events. Macropterous morphs are particularly important for habitat specialists such as *Ch. montanus* as they increase the probability to colonize new suitable habitats and to counteract anthropogenic habitat deterioration. Zera & Denno (1997) assumed that genetic as well as environmental conditions determine macropterism in Orthoptera. We raised single-sex groups of *Ch. montanus* in the laboratory under three different densities and two different temperature regimes. Afterwards, we measured body size, wing length, mortality and development time to test whether density or temperature affect nymphal development. The results did not confirm density- or temperature-induced macropterism in *Ch. montanus*, but a strong effect of temperature and density on other life history traits such as development time and growth rate was detected. Both sexes developed faster and attained larger body sizes under warmer conditions, but the effects of density under cool conditions differed among sexes. Males became smaller with increasing density, whereas in females development time increased. The results have been published in *The Biological Journal of the Linnean Society* (Rohde *et al.*, 2015).

Habitat fragmentation and habitat loss threaten *Ch. montanus* populations and lead to decreasing population sizes. Although macropterism is occasionally found in wild populations of *Ch. montanus*, it is a highly immobile insect species. Due to its small population sizes and strong habitat specialization it is susceptible to environmental changes. Particularly interspecific interactions such as hybridization may be caused or altered by environmental changes and are of high interest for conservation. In **Chapter II** it was tested if interbreeding between *Ch. montanus* and *Ch. parallelus* is related to heterospecific encounter probabilities and examined the prevalence of hybridization in 16 populations using a microsatellite analysis. We found that hybrids occur in all syntopic populations and that heterospecific encounter probability increases hybridization risk. Furthermore, this study shows that increasing hybridization frequency did not positively affect genetic diversity of *Ch. montanus*, but of *Ch. parallelus*. The results of this study are summarized in an unpublished manuscript.

In **Chapter III** the potential effects of climate and extreme climatic events on population dynamics, population structure and hybridization in two syntopic populations of *Ch. montanus* and *Ch. parallelus* are assessed. We studied population dynamics using mark-recapture analyses and recorded data on the exact GPS position and soil moisture of each individual. Additionally, genetic samples of each captured individual were taken. Based on the mark-recapture analysis the population development (population size and phenology) of all populations was estimated for a period of five years. In order to analyze the impact of weather on population dynamics, we tested for correlations between population size changes and weather data. We also calculated spatially explicit probabilities of *Ch. montanus* individuals to encounter *Ch. parallelus* in the occupied habitat, which could provide information on hybridization risk. The genetic analyses helped to identify hybrids and test their localities with respect to the heterospecific encounter probabilities of the preceding years. Altogether, the study provides new insight concerning the function of ecological and phenological reproductive barriers as well as their response to changing environmental conditions. The results are presented in an unpublished manuscript.

In most cases, anthropogenic hybridization caused by habitat destruction, invasive species and climate change represents an additional threat to endangered species and is therefore of concern for conservationists. A major question that arises is how to deal with hybridization and hybrids in environmental law? **Chapter IV** provides an overview of the historical background of hybrids and hybridization becoming a part of legal instruments in three levels of law (international, European, national). Furthermore, legal uncertainties dealing with these terms as well as possible challenges for new conservation guidelines are analyzed. The results are summarized in an unpublished manuscript.

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Chapter I

Sex-specific phenotypic plasticity in response to the trade-off between developmental time and body size supports the dimorphic niche hypothesis

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Published in **Biological Journal of the Linnean Society** (2015)

Abstract

Female-biased sexual size dimorphism (SSD) is widespread in many invertebrate taxa. One hypothesis for the evolution of SSD is the dimorphic niche hypothesis, which states that SSD evolved in response to the different sexual reproductive roles. While females benefit from a larger body size by producing more or larger eggs, males benefit from a faster development, which allows them to fertilize virgin females (protandry). To test this hypothesis, we studied the influence of temperature and intraspecific density on the development of *Chorthippus montanus*. We reared them in monosexual groups under different conditions and measured adult body size, wing length, nymphal mortality, and development time. The present study revealed an inverse temperature-size relationship: body size increased with increasing temperature in both sexes. Furthermore, we found intersexual differences in the phenotypic response to population density, supporting the dimorphic niches hypothesis. At lower temperature, female development time increased and male body size decreased with increasing density. Because there was no food limitation, we conclude that interference competition hampered development. By contrast to the expectations, mortality decreased with increasing density, suggesting that interference did not negatively affect survival. The present study shows that sex-specific niche optima may be a major trigger of sexual dimorphisms.

***Keywords**:* Bergmann’s rule; crowding; density dependence; latitudinal compensation hypothesis; life history traits; sexual size dimorphism; temperature size rule.

Introduction

It is a well-known phenomenon that the sexes of most biota differ fundamentally in morphology, ecology and behavior (Darwin, 1859). Sexual differences in size (i.e. sexual size dimorphism; SSD), are particularly widespread among animal species (Davidowitz, Nijhout, 2004; Fairbairn, 1997; Froehlich, 1994; Hedrick, Temeles, 1989; Honěk, 1993). In most cases, SSD is female-biased, although exceptions occur (particularly among birds and mammals; Andersson, 1994; Blanckenhorn, 2005). There are several hypotheses explaining the evolutionary origin of SSD (Hedrick, Temeles, 1989; Lande, 1980; Shine, 1989; Slatkin, 1984; Temeles *et al.*, 2000). The most widely studied one is the sexual selection hypothesis, which proposes that sexual dimorphism emerges due to competition for mates or mate choice (Hedrick, Temeles, 1989). It is assumed that sexual selection generally favors male-biased SSD based on the competition advantages for larger males during mate acquisition. Although female-biased SSD may also be a result of sexual selection (e.g. male preferences for larger females), ecological factors could also cause natural selection for sexual size dimorphism (Blanckenhorn, 2005; Crowley, 2000; Shine, 1989).

The role of natural selection for SSD is highlighted by the dimorphic niche hypothesis (or reproductive role hypothesis), which states that the differential reproductive roles of the sexes are associated with differential energetic costs (Savalli, Fox, 1998), leading to different fitness optima (Hedrick, Temeles, 1989; Hochkirch *et al.*, 2007; Shine, 1991; Slatkin, 1984). These different optima are described by differential equilibria of the three major selective forces:. sexual selection, fecundity selection and viability selection (Blanckenhorn, 2005). In females of most invertebrate species, fecundity selection is considered to select for larger individuals, favoring larger clutch size, egg size or offspring size (Honěk, 1993; Reeve, Fairbairn, 1999). Males may benefit from a smaller body size, favoring sooner adult emergence (i.e. protandry), which increases the potential to fertilize virgin females (e.g. Bidau, Martí, 2007; Hochkirch *et al.*, 2008). Other advantages for small males may be reduced ecological competition with females or greater mobility and agility facilitating the search for mates (Kelly *et al.*, 2008), as well as decreased predation risk and lower food requirements (Blanckenhorn 2000). By contrast, sexual selection usually favors larger males (Savalli, Fox, 1998; Wiklund, Kaitala, 1995). The effect of viability selection on SSD remains poorly studied, although size-specific effects of predation, thermoregulation etc., are often assumed to be important for constraining the potential size differences between the sexes (Bouteiller-Reuter, Perrin, 2005; Cox, Calsbeek, 2009). Hence, the differential effects of fecundity selection are usually considered to be the predominating power behind female-biased SSD (Cox, Calsbeek, 2009; Reeve, Fairbairn, 1999), whereas sexual selection is considered to support male-biased SSD (Blanckenhorn, 2005; Stillwell *et al.*, 2010). Female-biased SSD is common in a majority of insect species and in 95 % of Orthoptera species (Hochkirch, Gröning, 2008; Stillwell *et al.*, 2010).

Body size is generally a plastic character, being strongly affected by environmental factors, as well as genetic preconditions (Honěk, 1993). Food quantity and quality, as well as temperature, affect growth and development (Angilletta Jr, Dunham, 2003; Atkinson, 1994; Davidowitz, Nijhout, 2004). The temperature-size rule states that, at lower temperatures, ectothermic individuals grow slower but attain larger body sizes than at higher temperatures (Angilletta Jr, Dunham, 2003; Davidowitz, Nijhout, 2004). Therefore, insects face a trade-off and may respond to energetic limitations either by reducing their body size or by a prolonged developmental time. Phenotypic plasticity of body size can vary substantially between the sexes and can cause intraspecific variation (Stillwell *et al.*, 2010; Teder, Tammaru, 2005). Given that the dimorphic niche hypothesis applies, one would expect that the sexes differ in their response towards environmental stresses, such as competition or limited energy supply to maximize their fitness (Blanckenhorn, 2005). Although females should aim to reach a maximum body size also under energetic limitation (which they may only reach by a longer developmental time), males should reduce body size to reach adulthood earlier.

We tested this hypothesis by raising single-sex groups of the water meadow grasshopper, *Chorthippus montanus* (Charpentier, 1825), in the laboratory under two different temperature regimes and three different densities. We measured adult body size, wing length, nymphal mortality and developmental time to investigate whether density and temperature affect nymphal development of the sexes differentially. Similar to most other grasshopper species, *Ch. montanus* is graminivorous but not further specialized in its diet. Because grasses are generally abundant in its habitat, we assumed that the species is not limited in food supply. Therefore, we assumed that these insects are mainly affected by interference competition and provided a sufficient food and water supply. We expected that, if the dimorphic niche hypothesis applies, males would attain a lower adult body size under the lower temperature regime and higher densities, whereas females would require a longer time to reach adulthood.

Materials & Methods

Study species

Chorthippus montanus is a univoltine, hygrophilous grasshopper species, which occurs in wet habitat types such as marshes, peat bogs, wet grassland, and water meadows (Froehlich, 1994; Ingrisch, 1983b; Kleukers *et al.*, 1997). Nymphs hatch in April and May and become adult from end of July to end of September (Weyer *et al.*, 2012). It is widely distributed over Eurosibiria from Western Europe to Kamtchatka (Kleukers *et al.*, 1997). In the study region, it is restricted to wet grasslands and bogs at elevations above 400 m (Weyer et al., 2012). As typical for most grasshopper species (Hochkirch *et al.*, 2008), *Ch. montanus* shows a pronounced sexual size dimorphism: males reach an entire body length of 13-16 mm and females 17-24 mm (Maas *et al.*, 2002).

Sampling

A total of 478 first- and second-instar nymphs of *Ch. montanus* was collected from 19 June to 3 July near Hundheim (49°50'3.96" N; 07°09'57.37" E) and Muhl (49°40'8.73" N; 07°02'27.12" E) in the Hunsrueck mountains (Rhineland-Palatinate, Germany). To avoid any possibility of confusion with nymphs of the closely-related grasshopper *Ch. parallelus*, nymphs were sampled only at localities, where *Ch. parallelus* did not occur (based upon studies in the preceding years). They were sorted by sex and kept in plastic terraria (19.5 x 30 x 20.5 cm) containing grass for transport.

Experimental design

We performed a full factorial experiment with two factors: temperature (two factor levels: 22° C and 27° C) and population density (three factor levels: two, four and eight individuals). The grasshoppers were separated by sex but, because of uneven sample size, it was not possible to achieve an equal number of replicates for both sexes (Table 1). Initially, we started the experiment with 16 replicates per temperature and density, aiming to sustain ten replicates after replacement of dead individuals. Whenever initial density decreased as a result of mortality, dead insects were removed and replaced by grasshoppers from a terrarium of the corresponding factorial combination (i.e. similar temperature and population density), which was subsequently excluded. The final number of replicates for each factor level combination varied between twelve and 15 (Table 1).

Table 1: Initial number of replicates (terraria) per density (two, four or eight individuals per terraria) and temperature (22°C and 27°C) sorted by sex.

	22° C		**27° C**	
Density / Sex	Male	Female	Male	Female
2	8	9	7	7
4	8	9	8	9
8	7	10	6	10
Sum	23	28	21	26

Experimental terraria (15.3 x 23 x 16.5 cm) were covered with soil with grass seeds and sand for oviposition. The aeration was ensured with a mesh lid. Seed propagation took place under standardized conditions in climate chambers (22° C, 65 % relative humidity) with daily watering. The experiment took place in two climate chambers (Kälte Kamrath) under a 17: 7 h light/dark cycle at constant temperatures (22° C or 27° C) and a relative humidity 65%. Each terrarium was illuminated by two UV- and VIS emitting fluorescent tubes (Osram Biolux® L36W/965). The terraria were inspected daily (except for one day) until all specimens were adult (total period 51 days). Grass was cultivated in each terrarium and additionally fresh grass was added every second day to avoid food limitation. The number of individuals (dead insects, number of final molts) was noted. After final molt, body size (the length of the insect from the frontal ridge to the tip of the abdomen) and wing length (length of the elytron) of each grasshopper were measured using a caliper (with accuracy of 0.01mm/0.0005). For subsequent analysis, we calculated the mean values for each terrarium. It was also noted wether a specimen was macropterous (i.e. had fully developed fore and hind wings).

Statistical analysis

Three-way analysis of variance (ANOVA) was used to analyze the influence of the explanatory variables temperature, density and sex on the response variables body size, wing length, relative wing length (wing length/body size) and time of nymphal development. The time of nymphal development was defined as the time from collecting to the final molt. To analyze the effect of temperature and density on SSD, we chose terraria randomly and calculated the ratio of mean female length to mean male length for these terraria combinations (resulting in six to eight replicates per temperature-density combination). All response variables were Box-Cox-transformed using the MASS library for R to infer the optimal lambda (i.e. the exponent for each variable) to achieve an optimal data distribution for ANOVAs (Venables, 2002). We stepwise simplified the ANOVA models using the step function in R, which uses the Akaike Information Criterion (AIC) to remove non-informative interaction terms. When significant interactions with sex occurred, we also analyzed the data for each sex separately in ANOVAs. To illustrate potential correlations between variables, we performed two principal component analyses (PCA) in R using the VEGAN package, version 2.0-10 (Oksanen *et al.*, 2013). The PCAs were performed for each sex separately because the major body size differences between sexes would otherwise mask the effects of temperature and density. We scaled each factor by their proportional eigenvalue because of the strong variability of the scales of our data (density, temperature) with the PCA functions were tested for significance using environmental fitting with 1000 permutations. To test, whether the occurrence of macropterous individuals was affected by temperature or density, we used two- or three-sample tests for equality of proportions with continuity correction, respectively. All tests were carried out in R 3.0.2 (R Development Core Team, 2014).

Results

Body size and wing length

As typical for grasshoppers, females were significantly larger than males (ANOVA, $\lambda = -1.42$, $F_{1,86} = 983.0$, $P < 0.001$). Furthermore, temperature significantly influenced body size (ANOVA, $\lambda = -1.42$, $F_{1,86} = 44.0$, $P < 0.001$). At higher temperatures (27°C) individuals reached significantly larger sizes than at lower temperatures (Fig.1). We found no significant main effect of density on body size but a near-significant trend (ANOVA, $\lambda = -1.42$, $F_{2,86} = 2.88$, $P = 0.062$) and a significant interaction between sex, density and temperature (ANOVA, $\lambda = -1.42$, $F_{2,86} = 4.38$, $P = 0.015$, Fig.1). There was a near-significant trend for smaller relative wing lengths at higher temperatures (ANOVA, $\lambda = 1.25$, $F_{1,95} = 3.2$, $P = 0.077$). Only four macropterous individuals were found in the present study, all of which were females. Because of the small number of macropters, these individuals were excluded from the analysis of the wing length. Sexual size dimorphism showed no significant response to the two explanatory variables, although there was a

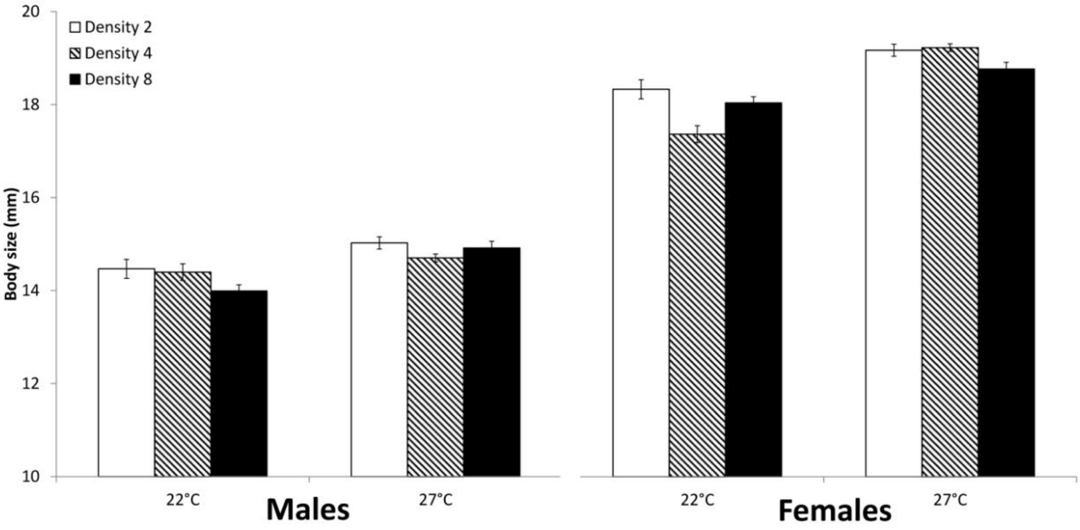

Figure 1 Mean adult body size of males and females of *Chorthippus montanus* at rearing temperatures of 22°C and 27°C and at densities of two, four and eight individuals per terrarium. Error bars are standard errors.

slight tendency of larger SSD at higher temperatures (22° C: 1.26 ± 0.02 SE; 27° C: 1.29 ± 0.01 SE; ANOVA , $\lambda = 3.9$, $F_{1,42} = 2.526$, $P = 0.12$).The PCAs for both sexes were highly similar (Fig. 2, but note that the loadings are reversed). The first function was mainly explained by growth rate (male score: 1.58; female score: -1.71), body size (male: 1.23; female: -1.55), and development time (male: -1.51; female: 1.61), whereas the second function was mainly explained by wing length (male: 1.62; female: -1.74) and relative wing length (male: 1.55; female: -1.67). The two first principal components explained 81% (males) and 88% (females) of the variance. Only temperature correlated significantly with both PCAs (environmental fitting: $P < 0.001$).

Effects on time of nymphal development and mortality

In both sexes, the developmental time took significantly longer at 22° C than at 27° C (ANOVA, $\lambda = 0$, $F_{1,93} = 113.8$, $P < 0.001$), although males generally developed faster than females, on average 2.5 days (ANOVA, $\lambda = 0$, $F_{1,93} = 9.91$, $P = 0.002$; Fig. 3). Furthermore, we found a near-significant trend that developmental time increased with increasing density (ANOVA, $\lambda = 0$, $F_{2,93} = 2.51$, $P = 0.087$). When the sexes were tested separately, this effect was significant for females (ANOVA, $\lambda = 0$, $F_{2,48} = 4.54$, $P = 0.016$) but not for males (ANOVA, $\lambda = 0$, $F_{2,38} = 0.03$, $P = 0.97$, Fig. 3).

Mortality decreased significantly with increasing density (ANOVA, $\lambda = 0.35$, $F_{1,91} = 23.90$, $P < 0.001$; Fig. 4), but this effect was smaller at the lower temperature regime resulting in a

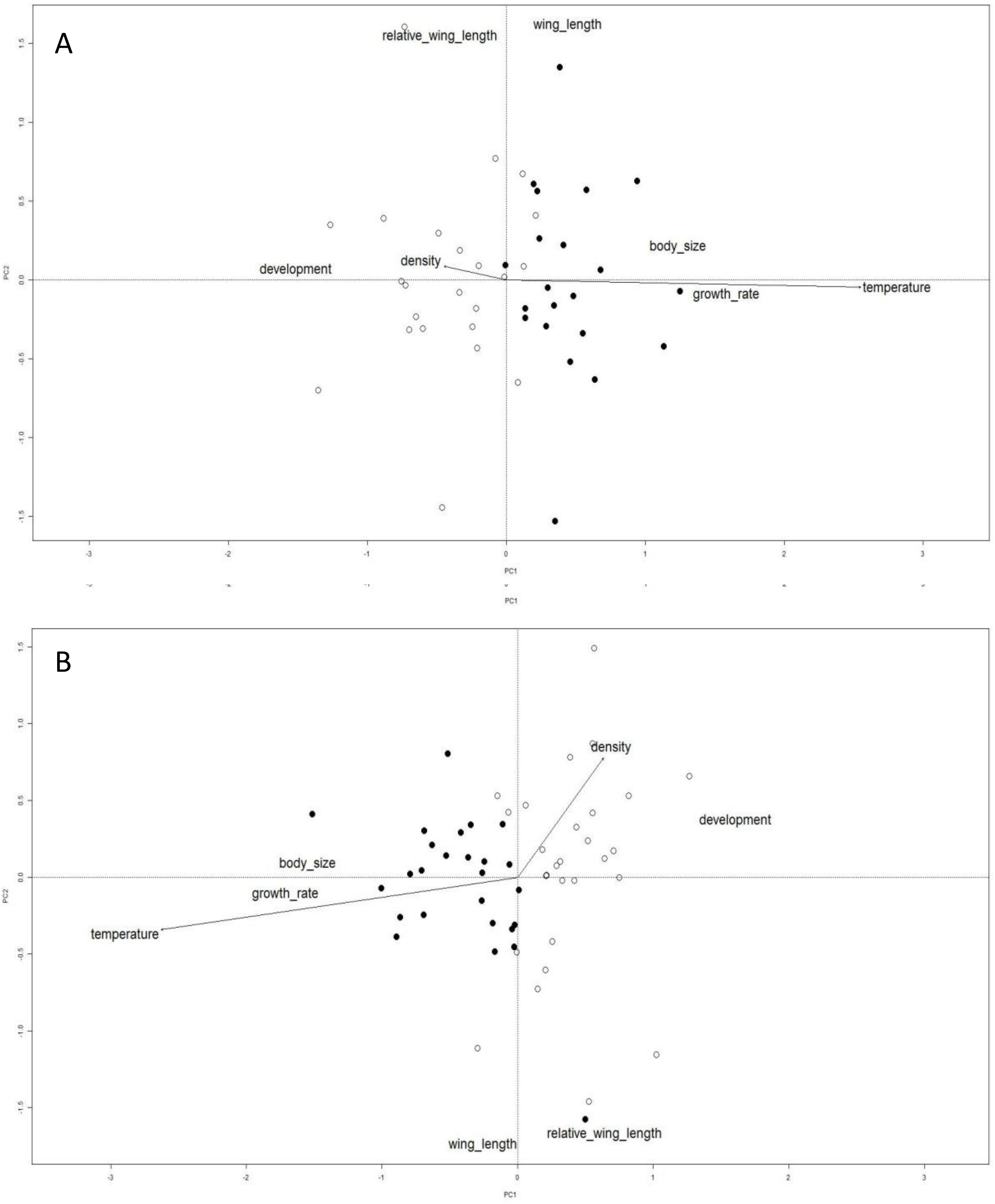

Figure 2. Plot of the first two functions of the principal component analyses (PCA) on the variables body size, development time, growth rate, wing length and relative wing length for males (a) and females (b), explaining 81% and 88% of the total variance for males and females, respectively. Each point represents one individual (black points: 27° C; white circles: 22° C). Arrows show the correlation of density and temperature using environmental fitting.

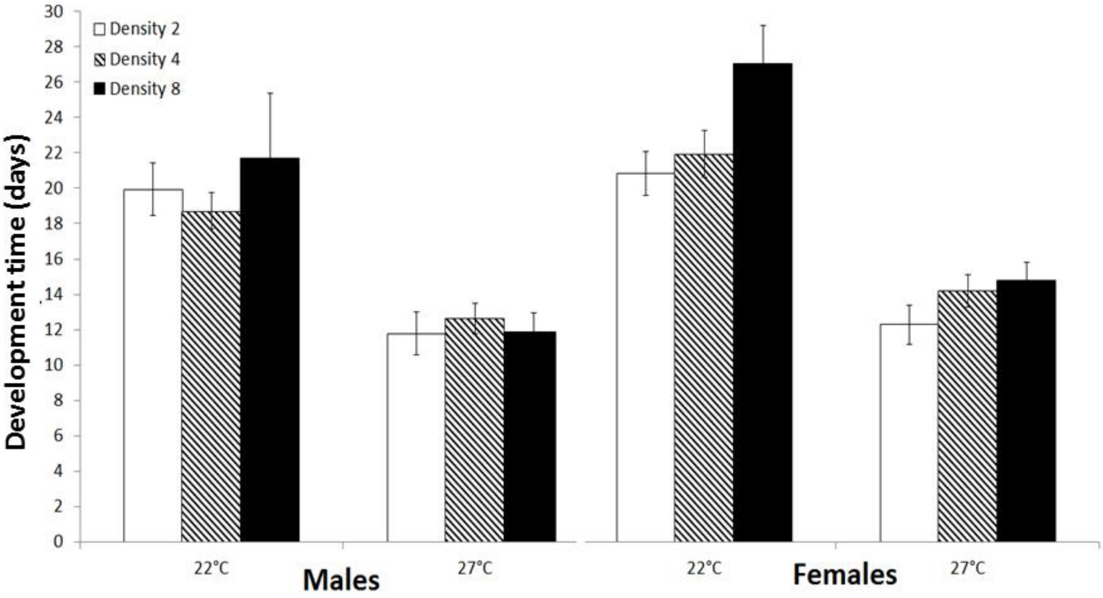

Figure 3 Development time of males and of females of *Ch. montanus* at rearing temperatures of 22°C and 27°C and at densities of two, four or eight individuals per terrarium. Error bars are standard errors.

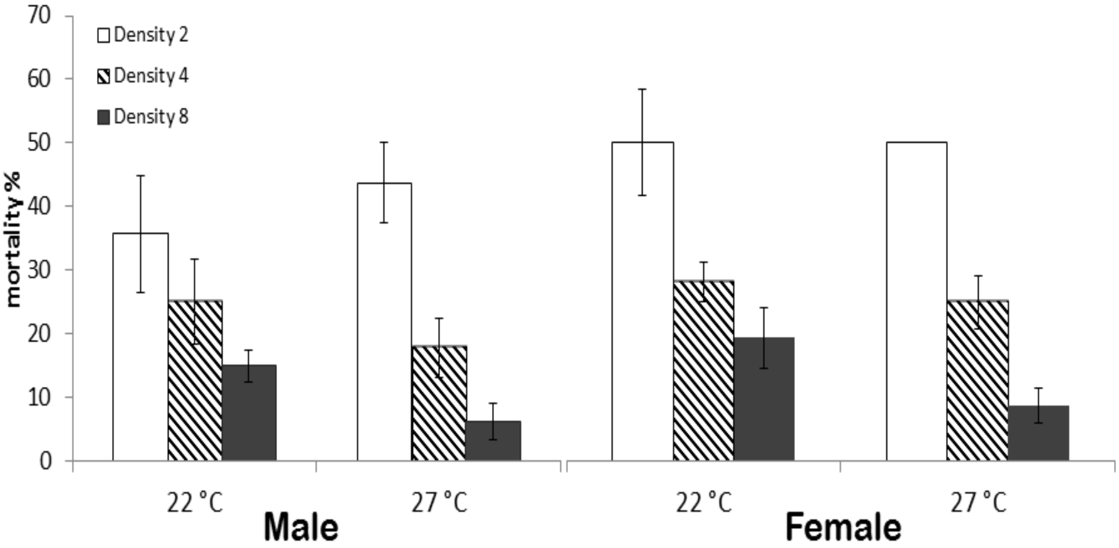

Figure 4 relative mortalitiy of males and and of females of *Ch. montanus* at rearing temperatures of 22°C and 27°C and at densities of two, four or eight individuals per terrarium.Error bars are standard errors.

significant interaction between temperature and density (ANOVA, $\lambda = 0.35$, $F_{1,91} = 6.58$, $P = 0.012$). Furthermore, mortality was significantly higher among females than among males but independent of experimental treatment (ANOVA, $\lambda = 0.35$, $F_{1,91} = 4.09$, $P = 0.046$, Fig. 4).

Discussion

The results of the present study show that the sexes respond differentially to increasing intrasexual density. By contrast to the temperature-size rule, the insects became generally larger but, in line with the rule, developmental time became shorter at higher temperature. Females generally took longer to reach adulthood when intrasexual density was higher, whereas, in males, developmental time was not affected by density. Instead, they obtained smaller adult body size at high densities but only when the temperature regime was lower (22° C). These results differ from those obtained by Wall & Begon (1987a), who showed that both sexes of *Ch. brunneus* have a prolonged development time and smaller weight at higher densities. Our results support the dimorphic niche hypothesis, which states that the major drivers of SSD are the different fitness optima of the sexes (Hedrick, Temeles, 1989; Hochkirch *et al.*, 2007; Slatkin, 1984). The sexes differ in their response to the trade-off between developmental time and body size and exhibit a sex-specific phenotypic plasticity. Females are considered to benefit from a large body size, which is necessary to produce a higher number of eggs and/or larger eggs, as has been shown by Hassall et al. (2006) for *Ch. brunneus*. They respond to competition by a prolonged nymphal development allowing them to attain an optimal body size for reproduction. This is in line with the results of Wall & Begon (1987b), who showed that, at higher densities, females benefit from larger body size by a higher reproductive success, whereas they did not find such a correlation at low densities. Furthermore, it was hypothesized that nymphal experience is more important than adult experience, which is in line with the results of the present study. By contrast, males may profit from a short nymphal development (protandry) and this allows them to fertilize virgin females at the beginning of the season. Hence, they respond to competition by reducing their body size to avoid a belated adulthood. It has been shown for other *Chorthippus* species (e.g. *Ch. biguttulus*) that females are highly reproductive during the first week of adulthood and often do not re-mate in their lifetime (Kriegbaum, 1988). This may differ in *Ch. montanus*, although females of its sibling species *Ch. parallelus* (which re-mate frequently) do not require fresh sperm to fertilize their eggs (Reinhardt *et al.*, 1999).

Competition

Because there was no food limitation in the present study, it is likely that interference competition (Case, Gilpin, 1974; Schoener, 1983) was the major factor driving the observed pattern. The effects of interference are mainly attributed to behavioral changes that ultimately lead to differences in energy and time management, which may be negatively correlated with growth (Goss-Custard, 1980; Huntingford, Turner, 1987). In our case, density negatively affected nymphal growth in both sexes, although only females delayed development. The underlying behavioral interactions remain somewhat dubious because, in contrast to dragonfly larvae (Johnson *et al.*, 1985) grasshopper nymphs are not territorial. Because we kept the sexes separated, it is also unlikely that the nymphs already showed sexual interest in other individuals (although one might suspect some incipient rivalry among male nymphs). Furthermore, the tested densities were probably not sufficiently high enough to invoke crowding effects in a strict sense, although it should be noted that crowding effects are typically graded (Applebaum, Heifetz, 1999). However, the observed response was reverse from crowding effects known from two other grasshopper species in which crowding accelerated development rather than slowing it down (Uvarov, 1977). We assume that the negative effects of density on nymphal development stem from the energetic costs of direct interactions and the associated loss of time left for feeding. Interestingly, mortality decreased with increasing density, whereas the opposite would be expected under typical crowding conditions (Joshi *et al.*, 1998; Wall, Begon, 1986). It might be suggested that the observed changes in the development of *Ch. montanus* compensate for the negative effects of interference, and even lead to an overcompensation. This is supported by Abrams et al. (1996), who found that increased growth rate might be associated with greater juvenile mortality.

Converse temperature-size rule

In the present study, the general effects of temperature were similar for both sexes (i.e. the insects became larger and developmental time became shorter at higher temperatures). These results support previous findings that most Orthoptera species follow a converse temperature-size rule (Eweleit, Reinhold, 2014; Laiolo *et al.*, 2013; Parsons, Joern, 2014; Whitman, 2008). This is in line with the latitudinal compensation hypothesis (Blanckenhorn, Demont, 2004; Parsons, Joern, 2014), stating that smaller body size at colder temperature is a response to time constraints for development as a result of short seasons (i.e. the insects need to complete their life cycle within one season). Higher temperatures allow a faster growth rate, shorter development time, and an earlier oviposition (Roff, 1980; Walters, Hassall, 2006). Another proximate explanation given by Walters & Hassall (2006) is that the performance of the temperature-size rule or its converse depends upon the minimum temperature thresholds for growth versus development. However, it is probably more appropriate to suggest differences in the temperature-relationships for both factors. If we compare the growth rates in our experimental treatments (by dividing adult body size by developmental time), it becomes obvious that growth rate is substantially reduced at lower temperature (by 43% across all densities and sexes) and higher density (up to 24% for females comparing the highest and lowest densities at the low temperature regime). Our results differ from those modelled by Abrams et al. (1996), who suggested that an increase time in available would result in an increased growth rate and/or reduced adult body size. However, because developmental time was strongly determined by temperature in the present study, the direct effects of temperature are difficult to separate from those mediated through developmental time. *Ch. montanus* thus follows a typical compensatory life history strategy, focusing on completing development within one season (Parsons, Joern, 2014), whereas expansion of the growth rate is only possible at higher temperatures. This is probably an adaptation to its cold environment. The species occurs in wet grasslands (with a permanently high water table) and also at higher latitudes (Kleukers *et al.*, 1997) and has one of the latest adult emergence period among Central European grasshoppers (Weyer *et al.*, 2012). In such a case strong selection towards completing the life cycle is likely. Indeed, other species of the genus *Chorthippus* also show a decrease in body size with increasing temperature (Laiolo *et al.*, 2013). Walters and Hassall (2006) suggest that the variable responses of ectothermic species are based on their enzyme kinetics associated with growth rate and development rate. Univoltine populations may benefit from an increasing body size with temperature as a result of direct fitness benefits in female fecundity (Laiolo *et al.*, 2013).

Macroptery

By contrast to other studies (Köhler, 2002; Poniatowski, Fartmann, 2009), we found no effects of density on the number of macropters. Although our maximum densities were slightly lower than in the previous studies, they were considerably higher than under natural conditions, with 229 ind. m^{-2} in the present study versus 0.1-7.3 ind. m^{-2} in the field (Ingrisch, Köhler, 1998; Weyer *et al.*, 2012). In Orthoptera, the most important phase during which macropterism is determined comprises the first two nymphal instars (Köhler, 2002). Because we caught the specimens during this phase, it seems unlikely that we missed the sensitive period of the nymphs, although it cannot be completely excluded. An alternative explanation for the lack of density-induced macroptery in the present study might be found in the strong habitat specialization of *Ch. montanus*. Macropterous individuals have a low probability to colonize suitable habitats as these are scarce and naturally fragmented (at least in Central Europe). Moreover, macropterous morphs typically have a lower fertility (Crnokrak, Roff, 1995; Fairbairn, Preziosi, 1996; Köhler, 2002; Roff, Fairbairn, 1991). The combination of these two properties may decrease the benefits of macropterism. However, in another habitat specialist, *Metrioptera brachyptera*, density-induced macroptery has been documented (Poniatowski, Fartmann, 2009), even though macropters are rarely found in nature in this species (Poniatowski, Fartmann, 2011a).

Despite the low propensity of *Ch. montanus* to develop into a macropterous morph, such alate specimens are regularly found in natural populations, and also in the present study, four individuals became macropterous. We thus hypothesize that genetic effects play a more important role for wing dimorphism in this species than environmental factors. The importance of a genetic propensity for macroptery has also been proposed for species that show a strong density-response of macropterism (Simmons, Thomas, 2004). Zera & Denno (1997) therefore assume that both genetics and environmental conditions are equally important for macroptery. However, it is likely that the response to environmental factors is highly variable among species and probably even among individuals.

Conclusions

The results of the present study show that nymphal development of *Ch. montanus* is strongly influenced by temperature and population density. *Ch montanus* follows the converse temperature-size relationship, as do other Orthoptera, which might be an adaptation to its cool habitat and northern distribution. The differential response of the sexes to increasing population density at low temperatures is in line with the dimorphic niche hypothesis and provides evidence for a sex-specific phenotopic plasticity in *Ch. montanus*. Although males attained smaller body sizes when reared at high densities, the females responded by a prolonged development. We hypothesize that this response maintains maximum fecundity, allowing females to produce a high number of eggs per clutch or larger egg sizes and males to become adult before female final molt with a higher chance to fertilize virgin females.

Acknowledgements

The present study was funded by the German Research Foundation (DFG, GRK 1319) in the interdisciplinary graduate school ‘Cooperation of Science and Jurisprudence in Improving Development and Use of Standards for Environmental Protection – Strategies for Risk Assessment and Management’.

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Chapter II

Wide prevalence of hybridization in a rare wetland grasshopper increases genetic diversity in its widespread congener

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Published in **BMC Evolutionary Biology** (2015)

(in press)

Abstract

Background: Hybridization between species is of conservation concern as it might threaten the genetic integrity of species. Anthropogenic factors can alter hybridization dynamics by introducing new potentially hybridizing species or by diminishing barriers to hybridization. This may even affect sympatric species pairs through environmental change, which so far has received little attention. We studied hybridization prevalence and the underlying behavioral mechanisms in two sympatric grasshopper species, a rare specialist (*Chorthippus montanus*) and a common generalist (*Chorthippus parallelus*). We conducted a mate choice experiment with constant intraspecific density and varying heterospecific density, i.e. varying relative frequency of both species.

Results: Mate choice was frequency-dependent in both species with a higher risk of cross-mating with increasing heterospecific frequency, while conspecific mating increased linearly with increasing conspecific density. This illustrates that reproductive barriers could be altered by environmental change, if the relative frequency of species pairs is affected. Moreover, we performed a microsatellite analysis to detect hybridization in twelve syntopic populations (and four allotopic populations). Hybrids were detected in nearly all syntopic populations with hybridization rates reaching up to 8.9%. Genetic diversity increased for both species when hybrids were included in the data set, but only in the common species a positive correlation between hybridization rate and genetic diversity was detected.

Conclusion: Our study illustrates that the relative frequency of the two species strongly determines the effectiveness of reproductive barriers and that even the more choosy species (*Ch. montanus*) may face a higher risk of hybridization if population size decreases and its relative frequency becomes low compared to its sister species. The asymmetric mate preferences of both species may lead to quasi-unidirectional gene flow caused by unidirectional backcrossing. This might explain why genetic diversity increased only in the common species, but not in the rare one. Altogether, the hybridization rate was much higher than expected for a widely sympatric species pair.

Keywords: genetic displacement, introgression, mate choice, microsatellite, outbreeding, Orthoptera, pre-mating barrier

Introduction

The impact of hybridization and the underlying mechanisms have become fascinating fields of research for evolutionary biologists and conservation biologists (Rhymer and Simberloff 1996, Hochkirch and Lemke 2011, Abbott et al. 2013). The causes and consequences vary among species. While natural hybridization is recognized as a significant evolutionary process (Seehausen 2004, Genovart 2009), anthropogenic hybridization is often negatively valued by conservation biologists (Rhymer and Simberloff 1996). However, the potential outcomes of hybridization probably do not differ between natural and anthropogenic scenarios. Hybridization can trigger speciation and could lead to new adaptations in a changing environment (Seehausen 2004, Salmon et al. 2005, Chunco 2014, Thornton and Murray 2014). It can increase genetic diversity if hybrids are fertile, niches are available and both parental species have a high fitness (Mallet 2005). Furthermore, hybridization could counteract negative effects of a small population size such as inbreeding depression (Arnold et al. 2008, Abbott et al. 2013) and could thus protect a species against extinction (Baskett and Gomulkiewicz 2011). However, hybridization can also trigger the collapse of populations (and species) by genetic displacement (Schulte et al. 2012) and thus the negative effects of hybridization on rare species dominate the discussion in conservation biology (Rhymer and Simberloff 1996, Wolf et al. 2001, Seehausen et al. 2008).

The main natural scenarios, in which hybridization takes place, are secondary contact zones of species after postglacial range expansions (Butlin and Hewitt 1985, Barton and Hewitt 1989, Hewitt 1993). Anthropogenic drivers of hybridization include habitat loss, breakdown of ecological barriers or introduction of non-native or domesticated species (Rhymer and Simberloff 1996, Huxel 1999, Allendorf and Lundquist 2003, Randi 2008, Seehausen et al. 2008, Crispo et al. 2011, Chunco 2014). Most studies on natural hybridization focus on parapatric species in secondary contact zones, whereas hybridization between widely sympatric species received only little attention (Barton and Hewitt 1989, Bettles et al. 2005, Buggs 2007, Hochkirch and Lemke 2011, Hasselman et al. 2014). Even though there is a recent increase in studies on speciation with gene-flow (e.g. (Feder et al. 2012)), it is often assumed that sympatric species have evolved reproductive barriers that allow them to coexist (Rundle and Schluter 1998, Hochkirch and Lemke 2011). However, sympatric species do not necessarily occur in syntopy, i.e. they might differ in habitat affiliation, and thus might show a micro-allopatric distribution with several local hybrid zones (mosaic hybrid zones). Allotopy can reduce the negative effects of hybridization, but also might evolve as a consequence of such negative effects (Gröning and Hochkirch 2008). Even natural hybrid zones are influenced by anthropogenic factors and may for example be moving as a response of local hybridization equilibria to global warming (Engler et al. 2013). Similar changes might occur for species pairs with allotopic distribution patterns, e.g. if ecological barriers break down due to habitat deterioration or alteration (Seehausen 2006, Taylor et al. 2006, Hasselman et al. 2014). It is thus of high interest to study the patterns of hybridization in species pairs which are widely sympatric but only locally syntopic.

We investigated the hybridization prevalence and the underlying behavioral mechanisms in two sympatric grasshopper species, a rare specialist (*Chorthippus montanus,* Charpentier, 1825) and a common generalist (*Chorthippus parallelus,* Zetterstedt, 1821), which occur sympatrically in large parts of Eurasia. While *Ch. montanus* is a habitat specialist occurring in permanently moist habitats, *Ch. parallelus* is a habitat generalist which occurs in a variety of grassland habitats (Detzel 1998). *Ch. parallelus* is well known as a model species for hybridization studies, forming one of the best studied hybrid zones with an Iberian subspecies in the Pyrenees (Butlin and Hewitt 1985, Butlin 1998). Previous studies have even shown that *Ch. parallelus* and *Ch. montanus* hybridize under laboratory conditions and that hybrids are fertile at least to the F2-generation (Bauer and von Helversen 1987, Hochkirch and Lemke 2011, Köhler 2013). Juvenile mortality of *Ch. montanus*♂ - *Ch. parallelus*♀ hybrids is 34% higher than in the parental species, while in *Ch. parallelus*♂ - *Ch. montanus*♀ hybrids it is even lower than in the parental species. Egg mortality is 10% lower in F1 hybrids and 16% lower in F2 hybrids than in the parental species (Köhler 2013). Both species are closely related and morphologically very similar, but differ in ecology (Reynolds 1980, Detzel 1998, Hochkirch and Lemke 2011). Their songs have a similar structure, but differ in speed (*Ch. montanus* sings slower) (Reynolds 1980, Bauer and von Helversen 1987, Hochkirch and Lemke 2011). Due to their close relationship and similar songs, and based upon the occurrence of intermediate phenotypes, hybridization has been suggested to occur in syntopic populations (Reynolds 1980). *Ch. montanus* is threatened by drainage of wetlands, abandonment of meadows, habitat fragmentation and increasing length of droughts (Weyer et al. 2012). During the last decades it has disappeared from nearly all sites < 400 m asl in our study region, suggesting that it may be strongly affected by climate change. It is thus of high interest to explore, whether hybridization might act as an additional threat for *Ch. montanus* and if it may increase in declining populations.

Hochkirch and Lemke (2011) demonstrated that females of *Ch. montanus* strongly prefer conspecific males as mates, whereas such a preference was not found for females of *Ch. parallelus* or males of both species. This may present at least a unidirectional pre-mating barrier which may reduce the hybridization probability between both species. However, it is well known that encounter rate is a major factor influencing mate choice and choosiness of females (Milinski and Bakker 1992, Gröning et al. 2007, Willis et al. 2011) and that previous exposure to heterospecifics may increase hybridization risk (Izzo and Gray 2011). Thus, we assumed that the encounter probability of heterospecific males strongly influences female mate choice also in *Ch. montanus* and that high heterospecific frequencies (i.e. skewed abundances) may trigger interspecific matings also between *Ch. montanus* females and *Ch. parallelus* males. We further hypothesized that the ongoing decline of *Ch. montanus* may increase heterospecific encounter probabilities and thus hybridization risk to increase with decreasing population size. In order to test this hypothesis, we first performed a mate choice experiment, in which we analyzed the role of heterospecific density on mate choice when conspecific density remains constant. We expected an increasing hybridization risk with increasing heterospecific frequency and a linear increase of conspecific matings with increasing conspecific frequency. As hybridization was only proven under laboratory conditions it also aimed to test for the prevalence and extent of hybridization in the field. Therefore, we performed a microsatellite analysis in twelve syntopic and four allotopic populations. In order to detect potential drivers of hybridization and to test the hypothesis that hybridization risk increases with decreasing population size, we analyzed the hybridization rates for correlations with effective population size. As there is a strong altitudinal pattern in the decline of *Ch. montanus*, we also tested for correlations of hybridization rate and altitude. Finally, we examined the impact of hybridization on the genetic diversity of both species (Schulte et al. 2012) in order to assess the direction of gene flow and to test for differences between the habitat specialist and the generalist.

Materials & Methods

Study species

Chorthippus montanus is a univoltine, hygrophilous grasshopper species, which occurs in moist habitat types such as marshes, peat bogs and water meadows (Ingrisch 1983, Froehlich 1994, Kleukers et al. 1997). The species is listed as threatened on red lists of several European countries (Weyer et al. 2012). In the study area (Fig. 1), it has a highly fragmented distribution and is mainly found on isolated wet meadows at altitudes >400 m asl. On most of these meadows *Ch. parallelus* occurs, too, but the latter species usually occupies drier areas surrounding the wet habitat of *Ch. montanus*. Nymphs of *Ch. parallelus* hatch earlier than those of *Ch. montanus* and become adult ca. one month earlier (Ingrisch and Köhler 1998, Hochkirch and Lemke 2011). Adults of both species co-occur at least over a period of two to three months. Both species are flightless, but occasionally macropterous individuals occur, which are believed to represent the main dispersal units (Kleukers et al. 1997, Detzel 1998). Hybrids of both species produce intermediate songs and are morphologically either intermediate or similar to *Ch. parallelus* (Hochkirch and Lemke 2011, Köhler 2013).

The collection of genetic samples and live specimen for this research was permitted by the “Struktur- and Genehmigungsdirektion Nord” Rhineland-Palatinate.

Mate choice experiment

Nymphs of *Ch. parallelus* were collected on 30 June, those of *Ch. montanus* on 06 August 2010 at three meadows: Prosterath (49°44'6.59"N; 06°54'12.87"E), Damflos (49°40'4.18"N, 06°59'33.52"E) and Hoxel (49°46'22.16"N; 07°06'9.44"E). Nymphs were reared in plastic terraria (30 x 19.5 x 20.5 cm) covered with soil and planted with grass, kept in climate chambers (Kälte Kamrath) at 25°C and 65 % RF. They were watered each day. Aeration was ensured with a mesh lid. Each terrarium was illuminated by two UV- and VIS emitting fluorescent tubes (Osram Biolux® L36W/965). Nymphs were raised in single species groups. Adult individuals were sorted out daily by species (based upon their morphology) and sex to ensure virginity (grasshoppers become sexually mature 1-2 weeks after final moult) and to ensure that females had no previous experience with any potential mates. Mate choice experiments took place in similar terraria with moist soil and grasses. We used a full factorial design with 40 replicates of four different factor levels (frequencies) for both species (Table 1). During each replicate we observed mate choice for 90 minutes at four different frequencies with one pair of the target species and either one, two, three or four heterospecific pairs (non-target pairs) (1:1, 1:2, 1:3, 1:4; Table 1). At each day, we conducted 3-9 replicates with randomly chosen factor levels. The terraria were inspected every five minutes (copulations last on average 37 min ranging from 15 to 90 minutes (Reinhardt 2001)) and all copulations were noted (time; type of copulation: target species conspecific, target female with heterospecific male, target male with heterospecific female, non-target species conspecific). Whenever a copulation occurred, the individuals involved were marked with a permanent non-toxic paint marker (Edding 780) and released in the terraria again to keep the density constant. After 90 minutes, we sorted unmated individuals by species and sex. These individuals were never used as target species again, but males were used as non-target species in other replicates to increase the frequency of heterospecifics. Mated individuals were kept in separate terraria to breed them for later experiments.

Table 1: Composition of the mate choice experiment

Target species	Frequency	*Ch. montanus*		*Ch. parallelus*	
		♀	♂	♀	♂
Ch. montanus	1:1	1	1	1	1
	1:2	1	1	2	2
	1:3	1	1	3	3
	1:4	1	1	4	4
Ch. parallelus	1:1	1	1	1	1
	1:2	2	2	1	1
	1:3	3	3	1	1
	1:4	4	4	1	1

Statistical analysis of the mate choice experiment

We analysed the effects of the explanatory variables (a) target species, (b) heterospecific density, (c) source locality and (d) time on the following response variables: (1) number of conspecific matings of the target species, (2) number of conspecific matings of the non-target species, (3) relative mating frequency of the non-target species (i.e. number of matings / pair), (4) number of interspecific matings with heterospecific males, (5) number of interspecific matings with heterospecific females, (6) time until first conspecific mating of both target and non-target species. For analysing the number of conspecific matings of the target species, we used generalized linear models (GLMs) with binomial data distribution. The number of conspecific matings of non-target species was also analysed with GLMs, but with Poisson distribution. We stepwise simplified all GLMs using the "step" function in R. As the number of interspecific matings was rather low, we analysed these data either with χ^2 tests or Fisher's exact tests (if the expected values were < 5). The relative mating frequencies and the time until the first conspecific mating occurred were analysed with ANOVAs. The data were Box-Cox-transformed to infer the optimal exponent (λ) to fit the data to the models assumptions. All statistical analyses were computed in R 3.1.1 (R Development Core Team 2014).

Table 2: Geographic coordinates of each sample location in the Hunsrueck mountains (in decimal degrees; coordinate system WGS84), abbreviations of each location and altitude (in meters).

Location	Abbreviation	X-coordinate	Y-coordinate	altitude
Siesbach	S.	7.226888	49.73729	456
Hochscheid	H.	7.217074	49.875070	507
Zuesch	Z.	7.010876	49.650941	509
Allenbach	Ab.	7.166868	49.754453	500
Muhl	M.	7.041020	49.671145	604
Hundheim	Hd.	7.152509	49.834350	473
Abtei	A.	6.966467	49.690865	500
Reinsfeld1	R1	6.883199	49.674076	480
Reinsfeld2	R2	6.899559	49.686529	525
Farschweiler	F.	6.827721	49.718864	392
Damflos	D.	6.984930	49.666523	540
Prosterath	P.	6.903598	49.735398	404
Gonzerath	G.	7.115982	49.863947	439
Ochsenbruch	O.	7.064372	49.694968	645
Börfink	B.	7.070153	49.685788	559
Hunolstein	Hust.	7.043359	49.802859	600

Genetic analyses- Data collection

In 2009 and 2010 we sampled 1159 specimens (570 *Ch. montanus*, 561 *Ch. parallelus* and 28 intermediate morphotypes) from 16 localities in the Hunsrueck Mountains, Rhineland-Palatinate, Germany (Table 2, Fig. 1). We removed single hind legs of about 40 individuals per population and species. On twelve of these localities both species occurred syntopically, whereas Ochsenbruch represents a pure *Ch. montanus* population. In this case, we collected *Ch. parallelus* from a meadow in close vicinity. The localities Hunolstein and Abtei represent a pure populations of *Ch. parallelus* and *Ch. montanus*, respectively, from which we only collected the respective species, because the sibling species did not occur in close vicinity. We removed single hind legs of about 40 individuals per population and species. On twelve of these localities both species occurred syntopically, whereas Ochsenbruch represents a pure *Ch. montanus* population. In this case, we collected *Ch. parallelus* from a meadow in close vicinity. The localities Hunolstein and Abtei represent a pure populations of *Ch. parallelus* and *Ch. montanus*, respectively, from which we only collected the respective species.

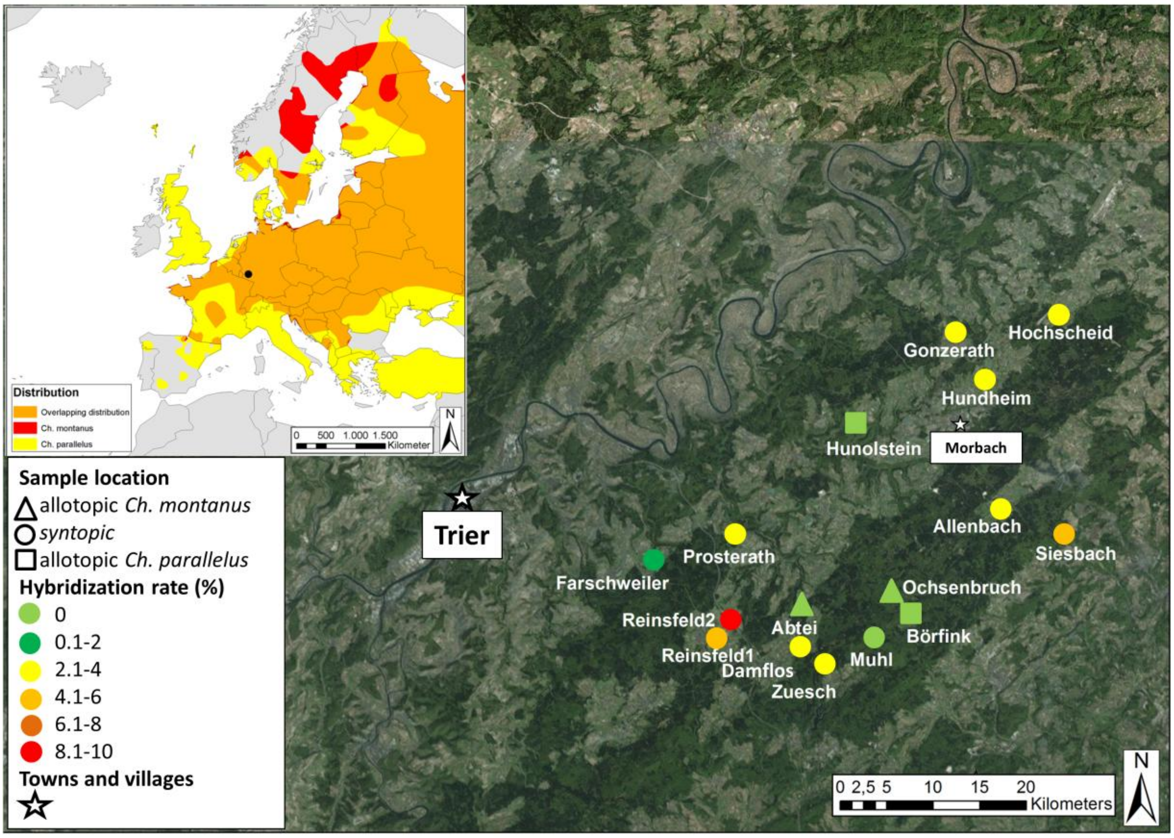

Figure 1 Top left: Distribution of *Ch. montanus* (red), *Ch. parallelus* (yellow) and the overlapping distribution of both species (orange) (modified after Kleukers 1997). Geographic map of each sample location in the Hunsrueck mountains and hybridization rate at each sample location (for geographic coordinates see Table 2). Triangle: allotopic population of *Ch. montanus*; square: allotopic population of *Ch. parallelus*; circle: syntopic populations.

Genotyping

DNA was extracted from hind femur muscle using the DNeasy Blood & Tissue Kit (Qiagen). All individuals were genotyped at ten polymorphic microsatellite loci. Six microsatellite markers were designed for *Ch. parallelus* (BF1, BD5, BH5, BD7, BF9, CD6; Molecular EcologyResources Primer Development Consortium Abercrombie *et al.*, 2009), four were developed for *Ch. montanus* prior to this study (CM5, CM19; CM 33; CM 37; Table 3).

For PCR we used the Qiagen Multiplex Mastermix in multiplexed PCR protocols for a combination of two to four loci with the following annealing temperatures (BF1, BH5, CD6, CM37: 54°C; BD5, CM5: 48°C; CM33, CM19: 51°C; BD7, BF9: 58°C). PCR tubes were filled with 10 µl reaction mixes (5.5 µl MultiplexMasterMix, 2 µl water, 1.4 µl genomic DNA (2-10 ng), 1.1 Primermix (1 µM / primer). The amplification was performed in a Multigene Gradient Thermal Cycler (Labnet) with the following PCR conditions: Initialization: 94°C/10min; Denaturation: 94°C/45sec; Annealing: see Primer/45sec; Extension: 72°C/45sec; Final Extension: 72°C/30min; 37 cycles. Each forward primer was labeled with a fluorescent dye (FAM, HEX or TAMRA). Fragment lengths of PCR products were determined on a MEGABACE 1000 automated sequencer (GE Healthcare) and scored with Fragment Profiler 1.2 (Amersham Biosciences).

Table 3: Characterization of four polymorphic microsatellite primers for *Ch. montanus* with: locus name; repeat motif; primer sequence of forward (for) and reverse (rev) primer; allele size range (bp) and fluorescence dye name (Tag).

Locus	Repeat motif	Primer-Sequenz 5´-3´	Allele size range (bp)	Tag
CM 5	(ATC)21	for: TGTACCCATGAGCTACTGTCA rev: TGGCAAACTGGCGAGCTTCT	306-432	HEX
CM 19	(TCTG)4(TCCG)3	for: CGATCGCCTTTTGACAGCTC rev: CCATATTCTCGCGTGGCTTG	410-450	FAM
CM 33	(GAT)11	for: ACAAACTGTCTCGAATACTTGC rev: GGTAGTAGCTATTCTTGAGTTG	301-349	TAMRA
CM 37	(TCA)6	for: GTTTCCGTGATCCTGAGCG rev: AGGTACTTGGATTCGGTGAG	219-339	TAMRA

Simulating and detecting hybrids

In order to detect hybrids in the data set, we simulated hybrids in HYBRIDLAB 1.1 (Nielsen et al. 2006). This simulation was based upon a subset of 120 purebred individuals of each parental species, which were chosen from the complete data set after discarding potential hybrids discovered in a preliminary analysis using three different programs. For the preliminary analyses we used STRUCTURE 2.3.4 and NewHybrids (which represent a Bayesian approach) and the R package adegenet 1.4-1 (which uses a discriminant analysis) (Anderson, Thompson, 2002; Jombart, 2008; Pritchard *et al.*, 2000). The Structure analysis was run with the admixture model, a burn-in of 10^4 simulations followed by 10^5 Markov chain Monte Carlo (MCMC) simulations and a K of two with ten iterations. The posterior probability (q) belonging to one of the two clusters was used to identify hybrids. The threshold q-value for hybrids was chosen between 0.2 and 0.8, as the simulation showed that a broader range led to an overestimate of hybridization caused by a higher number of mis-assigned pure-bred individuals, F1 and F2 hybrids (Fig. 2). Hence, the threshold used here represents a conservative estimate of hybridization as has also been shown in other studies (Schulte et al. 2012). NewHybrids was developed to detect hybrids and distinguish different hybrid classes (i.e. F1, F2 and backcrosses (Anderson and Thompson 2002)). The probabilities of each individual to belong to one of these hybrid classes were summed up and they were assigned to three categories based upon the maximum probability (i.e. either *Ch. parallelus*, *Ch. montanus* or hybrid). Posterior distributions were evaluated after 10^5 iterations of the MCMC and a burn-in period of 10^4 iterations. The third program adegenet 1.4-1 assigns genotypes to clusters based upon a discriminant analysis (DA), differentiating between hybrid classes. In this case, a prior assignment of all individuals to the classes is necessary. Therefore, individuals were assigned to a prior hybrid class, if this was suggested by both STRUCTURE and NewHybrids (only for the simulation study). The classification test assigned 90% of the genotypes correctly (Jombart 2008).

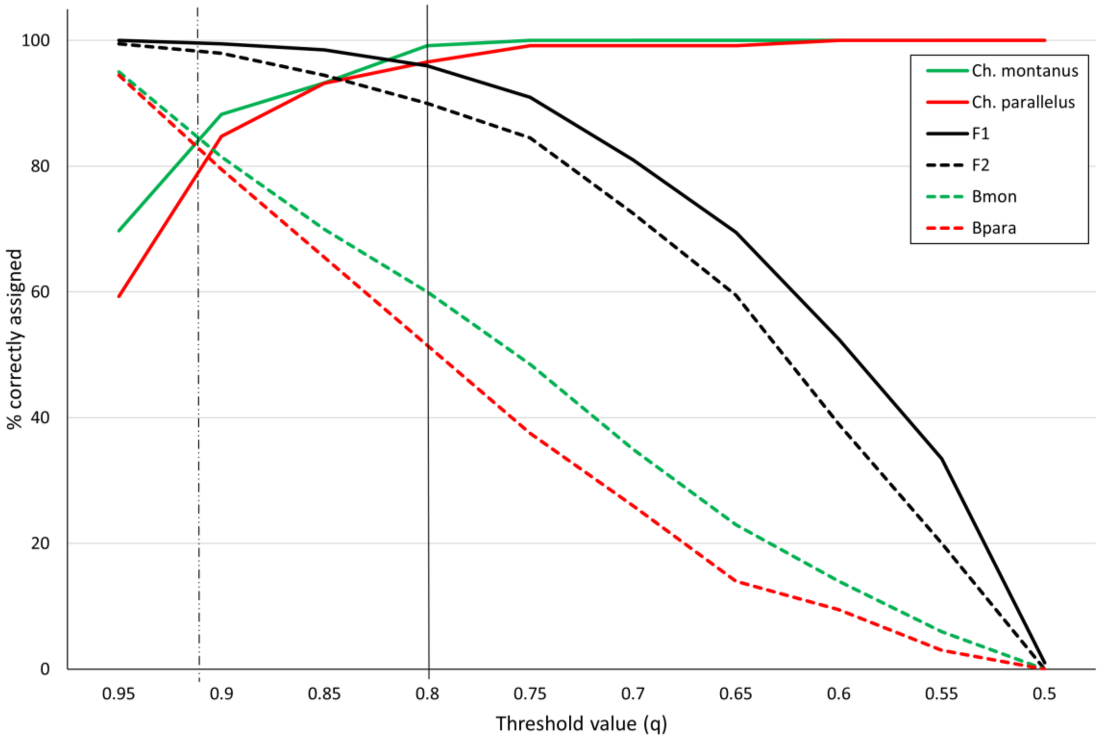

Figure 2 Mean number of conspecific copulations for target females of *Ch. montanus* and *Ch. parallelus* **(a)** with increasing heterospecific density, **(b)** with increasing intraspecific density, **(c)** Mean number of interspecific copulations for target females of *Ch. montanus* and *Ch. parallelus* with increasing heterospecific density (error bars are standard errors).

We simulated four classes of hybrids (F1, F2 and backcrosses with both species) with 200 individuals of each class in HYBRIDLAB 1.1 (Nielsen *et al.*, 2006). HYBRIDLAB allows a maximum of 120 individuals or individuals of each parental species to be included. Therefore, we first excluded all individuals identified as potential hybrids by at least two of the abovementioned programs. We then first included all individuals, which were collected from allotopic populations. The rest of parental individuals were randomly chosen from the dataset of purebred parental species. After simulating the hybrid classes, they were added to the dataset of parental individuals and the three abovementioned programs were used to determine the accuracy of hybrid detection by the different programs using the same settings.

The original dataset was then analyzed again using STRUCTURE, NewHybrids and adegenet (with the abovementioned conditions). Each individual was finally assigned to one of three classes: (1) *Ch. parallelus*, (2) *Ch. montanus*, (3) hybrid (including F1, F2 and backcrosses) using two different approaches: In the conservative assignment, we only assigned individuals as hybrids when they were detected by all three programs. In the relaxed assignment, we assigned individuals as hybrids when they were identified by at least two of the three programs. These two approaches were used to calculate the hybridization rate for each population (hybridization rate = N_h / N * 100; N = Total sample size of *Ch. montanus* and *Ch. parallelus* per population, N_h = Number of detected hybrids). The conservative approach was used for any further analyses, whereas the relaxed approach was just calculated to obtain an upper estimate.

Genetic diversity

Expected and observed heterozygosities (H_E and H_O) were calculated using GenAlEx 6.5 (Peakall and Smouse 2012). The mean number of alleles per locus (A) and allelic richness (A_R) were analyzed in Fstat 2.9.3.2 (Goudet 2001). These values were first calculated for each population of each species after excluding all hybrids detected by the conservative approach. In order to analyze the influence of hybrids on the genetic diversity of the populations, we performed a second analysis, in which we included the hybrids by assigning them to the parental population for which they had the highest assignment probability. In order to test for differences in genetic diversity in datasets with and without hybrids for each species, we only included populations where hybrids were detected and performed a paired t-test in R 3.0.2 (R Development Core Team 2014). Furthermore, allele frequencies, inbreeding coefficient (F_{IS}) and tests of Hardy-Weinberg-Equilibrium (HWE) were calculated in GenAlEx 6.5 (Peakall and Smouse 2012). Fixation indices for genetic differentiation (F_{ST}) between all populations of one species as well as between both species within syntopic and allotopic populations were also calculated in GenAlEx 6.5. Linkage disequilibria (based on 900 permutations and a nominal level of 1/100) between all pairs of loci were tested for each population of both species using Fstat 2.9.3.2 (Goudet 2001).

Correlation analyses

A linear regression analysis (lm) was performed in R 3.0.2 to analyze the relationship between the genetic parameters (A, A_R, H_o, H_e) of the populations (including hybrids) and hybridization rate. As we expected a higher hybridization probability with decreasing population size (based upon the mate choice experiment), we also calculated a linear regression between effective population size and hybridization rate. Effective population size (N_e) was calculated for each population and species using ONeSAMP1.2 (Tallmon et al. 2008). Here we used the datasets without potential hybrids (based upon the conservative approach) to avoid an artificial overestimation of the population size caused by the inclusion of hybrids. Finally, we analyzed the correlation between hybridization rate and altitude of the twelve syntopic populations (Table 2), because *Ch. montanus* went extinct at localities < 400 m during the last decades.

Results

Mate choice experiment

The complete number of copulations was similar among species (*Ch. montanus*: 150, *Ch. parallelus*: 155). Relative mating frequency of non-target pairs remained more or less constant among treatments (mean: 0.28 ± 0.02) and was not significantly affected by density or species. Altogether, we observed 34 interspecific matings (26 between *Ch. montanus* males and *Ch. parallelus* females and eight between *Ch. parallelus* males and *Ch. montanus* females). The number of conspecific matings of the target species did not differ significantly between species. However, it decreased in both species significantly with increasing density of heterospecifics (GLM, Rd = 196.4, df = 316, z = -4.02, p < 0,001; Fig. 3a).

The number of conspecific matings of the non-target species was also similar between species, but for both species the number of matings increased with increasing number of conspecifics (GLM, Rd = 250.9, df = 318, z = 7.41, p < 0,001; Fig. 3b). Target females of *Ch. parallelus* were more often involved in interspecific matings (12 x) than

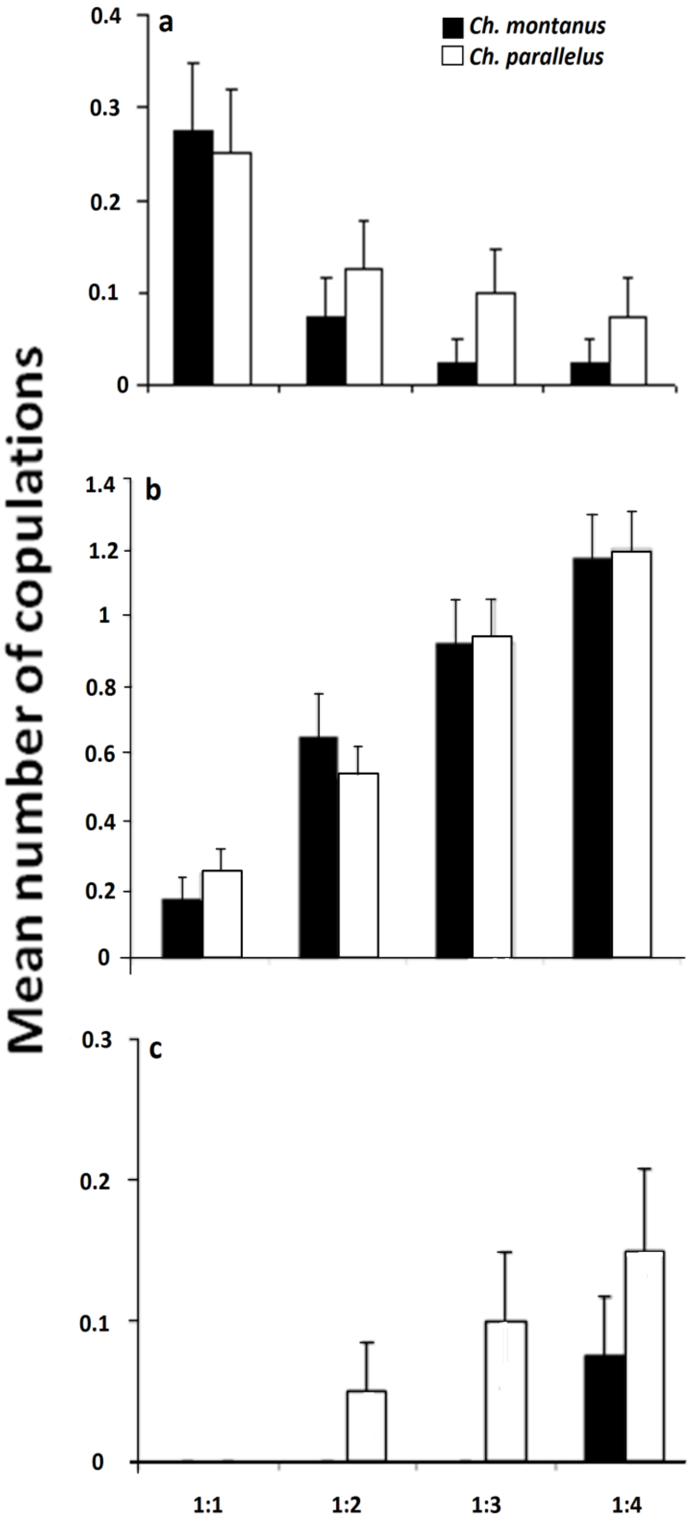

Figure 3 Mean number of **(a)** conspecific copulations for target females with increasing heterospecific density, **(b)** conspecific copulations for non-target females with increasing intraspecific density, **(c)** interspecific copulations for target females with increasing heterospecific density (error bars are standard errors).

those of *Ch. montanus* (3 x; χ^2 test, df = 1, $\chi^2 = 4.48$, $p = 0.034$; Fig. 3c), whereas the opposite was true for males (14 x for *Ch. montanus* males, 3 x for *Ch. parallelus* males; χ^2 test, df = 1, $\chi^2 = 6.21$, $p = 0.013$). Interspecific matings of *Ch. montanus* target females were not significantly affected by density (Fisher's Exact Test, $p = 0.059$), but only occurred at a density of 1:4, whereas in *Ch. parallelus* females the number of interspecific matings increased significantly with increasing heterospecific density (Fisher's Exact Test, $p = 0.045$; Fig. 3c). For males, no significant effects of heterospecific density on interspecific mating frequency were found (Fisher's Exact Test, *Ch. montanus*: $p = 0.47$, *Ch. parallelus*: $p = 0.99$). The time until a mating occurred varied between 28 and 65 minutes and was not significantly influenced by either density or species (ANOVA, *Ch. montanus*: $\lambda = 0.64$, $F_{1,71} = 0.67$, $p = 0.42$; *Ch. parallelus*: $\lambda = 0.5$, $F_{1,69} = 0.84$, $p = 0.36$).

Hybridization rate

After simulating a total of 800 hybrids (F1, F2, backcrosses with *Ch. montanus* and *Ch. parallelus*) in HYBRIDLAB, we tested the performance of the three programs by evaluating their assignement of the simulated hybrids. The program NewHybrids had the best performance with an accuracy of 90 %, when hybrids were assigned to the respective habrid class at an estimated posterior probability > 0.5. Adegenet detected 88 % of the simulated hybrids correctly and STRUCTURE detected 82 % at a q value between 0.2 and 0.8 (Fig. 4a).

When we performed the same analysis with the original dataset (excluding simulated hybrids), we detected 34 hybrids using the conservative approach. With the relaxed approach we identified 79 hybrids, i.e. 46 hybrids were detected by only two programs, 23 of which were assigned as backcrosses with one of the parental species by NewHybrids and adegenet. In STRUCTURE, we assigned these individuals as purebred species at the chosen threshold of $q > 0.8$ (Fig. 4b).

The hybridization rate of all tested populations varied between 0 and 8.9% for the conservative approach and between 0 and 14.4% for the relaxed approach (Table 3). The highest hybridization rates were found in the populations Reinsfeld1 (conservative: 6.0; relaxed: 10.84) and Reinsfeld2 (conservative: 8.9; relaxed: 14.44). In the relaxed approach, eight hybrids were also detected in the allotopic populations Ochsenbruch and Börfink, suggesting that this approach provides an overestimate. These hybrids were assigned as backcrosses with *Ch. montanus* (3x) for Ochsenbruch and *Ch. parallelus* (5x) for Börfink.

No hybrids were detected in the other allotopic populations (Hunolstein and Abtei) in any analysis.

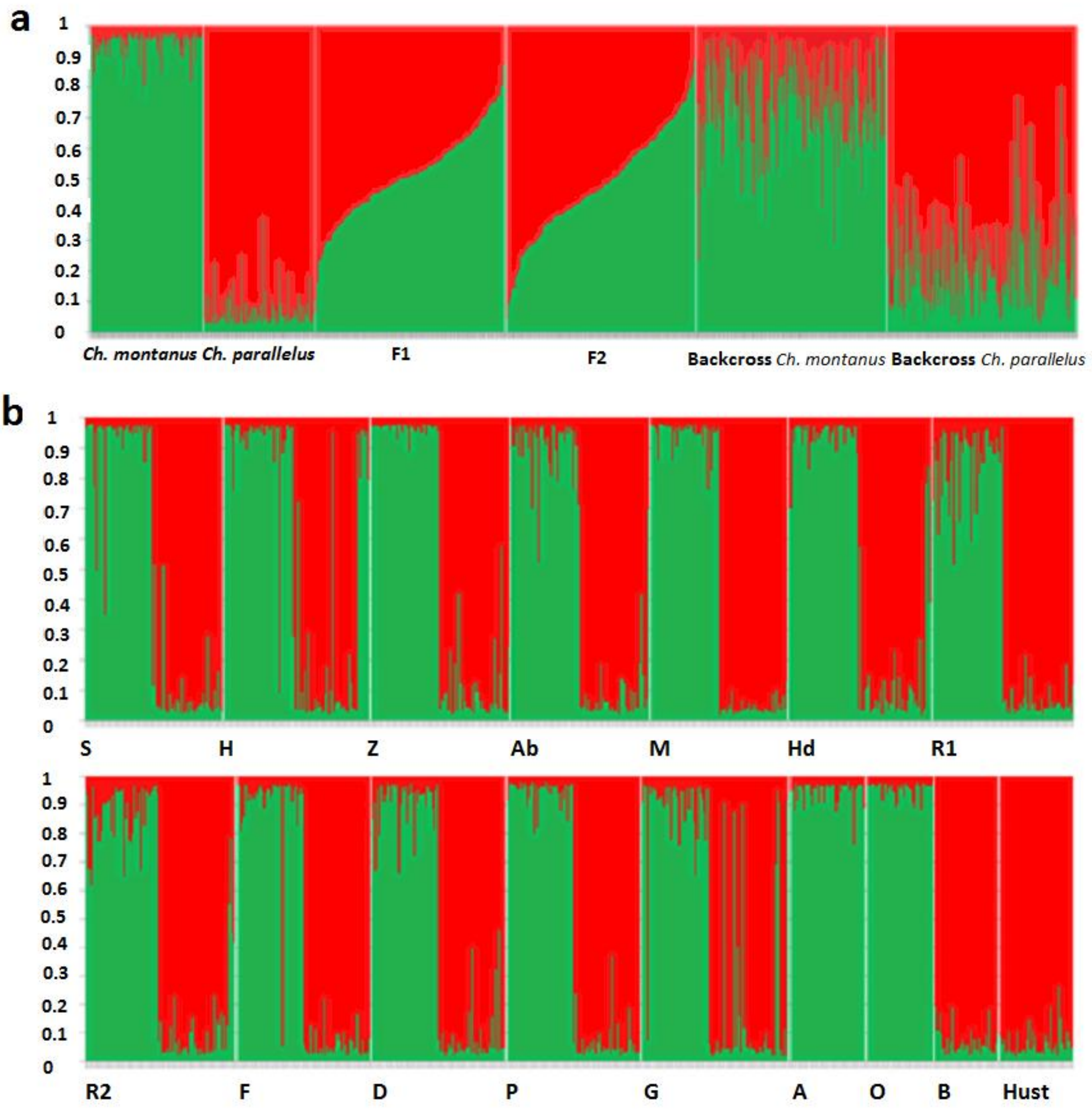

Figure 4 Genetic clusters found by STRUCTURE for (a) the simulated hybrid-classes and (b) the 16 sampled populations. Each individual is represented by a single vertical line, divided into K colours (K = 2; *Ch. montanus*: green; *Ch. parallelus*: red); the coloured segment shows the individual's estimated proportion of membership to that genetic cluster; abbreviations correspond to **(a)** the simulated hybrid-classes and to **(b)** the 16 sampled populations. The STRUCTURE run was performed with 10^5 MCMC and a burn-in period of 10^4 chains (with 10 iterations for each K). Populations O and A. were allotopic populations of *Ch. montanus*, B and Hust. were allotopic populations of *Ch. parallelus.*

Table 4: Number of hybrids detected in each population using STRUCTURE, NewHybrids and Adegenet (conservative estimate: hybrids detected by all three programs, relaxed estimate: hybrids detected by two programs), hybridization rate (in %) and sample sizes for each population and species (pre-identified by morphology).

Pop	Sample size *Ch. montanus*	Sample size *Ch. parallelus*	No. of hybrids conservative	No. of hybrids relaxed	Hybridization rate conservative (%)	Hybridization rate relaxed (%)
Abtei	45		0	0	0	0
Allenbach	40	39	2	5	2.5	6.17
Börfink		39	0	5	0	11.63
Damflos	40	39	2	6	2.5	7.5
Farschweiler	40	39	1	5	1.3	6.25
Gonzerath	43	43	2	6	2.3	6.82
Hochscheid	47	36	2	4	2.4	4.71
Hundheim	41	39	3	7	2.4	8.54
Hunolstein		44	0	0	0	0
Muhl	40	40	0	0	0	0
Ochsenbruch	40		0	3	0	6.98
Prosterath	40	38	2	7	2.5	8.75
Reinsfeld1	36	42	5	9	6	10.84
Reinsfeld2	40	42	8	13	8.9	14.44
Siesbach	38	38	5	6	5.8	6.98
Züsch	40	39	2	3	2.5	3.7
total	570	557	34	79	3.35	8.15

Genetic variability and diversity

The mean number of alleles was 11.44 ± 0.44 for *Ch. montanus* (excluding hybrids of the conservative assignment). Including the hybrids increased the mean number of alleles significantly by 8.3% (paired t-test: t= -3.9, df= 10, P= 0.003; Table 4). Similarly, the number of alleles in *Ch. parallelus* populations increased from 15.6 ± 0.63 by 5.3% when hybrids were included (paired t-test: t = -4.68, df = 10, P = 0.001; Table 5). When including hybrids, expected heterozygosity (H_e) declined significantly by 1.04% for *Ch. parallelus* (paired t-test: t = 3.89, df = 10, P = 0.003), but increased (not significantly) by 1.2% for *Ch. montanus* paired t-test: t = -2.09, df = 10, P = 0.064; Tables 4, 5). There was no significant difference in observed heterozygosities (H_o) between the datasets with and without hybrids (Tables 5, 6).

For some loci species-specific alleles were evident, e.g. in locus CM33 alleles 298-313 were common in *Ch. parallelus* but rare in *Ch. montanus*, while alleles 316-328 were common in *Ch. montanus* and rare in *Ch. parallelus*.

Table 5: Genetic parameters of each *Ch. montanus* population with hybrids (+) and excluding hybrids detected with the conservative approach (-)*;* N: sample size; A: mean number of alleles; H_o and H_e, observed and expected heterozygosities; N_e: mean effective population size estimate; numbers in parentheses are standard errors.

Pop	**N +**	**N -**	**A +**	**A -**	**Ho +**	**Ho -**	**He +**	**He -**	**Ne -**
S.	41	37	9.9 (1.4)	6.7 (1.1)	0.57 (0.09)	0.59 (0.09)	0.69 (0.09)	0.64 (0.09)	40.3
H.	47	46	13.2 (1.5)	12.9 (1.5)	0.66 (0.06)	0.67 (0.06)	0.81 (0.03)	0.81 (0.03)	667.7
Z.	41	40	12.0 (1.7)	11.0 (1.6)	0.69 (0.07)	0.70 (0.07)	0.80 (0.04)	0.80 (0.04)	142.7
Ab.	40	38	12.4 (1.6)	11.8 (1.6)	0.65 (0.06)	0.65 (0.06)	0.80 (0.04)	0.79 (0.04)	512.2
M.		38		12.2 (1.8)		0.71 (0.05)		0.80 (0.03)	389.4
Hd.	42	40	12.5 (1.6)	11.5 (1.7)	0.65 (0.06)	0.66 (0.06)	0.80 (0.04)	0.79 (0.04)	267.9
A.		44		10.7 (1.6)		0.60 (0.06)		0.78 (0.03)	122.3
R1	39	35	11.5 (1.6)	10.7 (1.5)	0.67 (0.06)	0.67 (0.06)	0.79 (0.03)	0.78 (0.03)	128.4
R2	46	38	11.9 (1.7)	11.1 (1.6)	0.57 (0.07)	0.59 (0.08)	0.75 (0.06)	0.75 (0.05)	170.9
F.	40	39	11.8 (1.7)	11.1 (1.6)	0.63 (0.06)	0.63 (0.06)	0.75 (0.04)	0.75 (0.05)	184.1
D.	39	37	13.9 (2.0)	13.3 (2.0)	0.63 (0.06)	0.63 (0.07)	0.79 (0.04)	0.79 (0.04)	656.5
P.	38	36	12.2 (1.7)	11.0 (1.6)	0.60 (0.07)	0.59 (0.07)	0.78 (0.05)	0.77 (0.05)	295.9
G.	44	42	14.7 (1.9)	14.5 (1.8)	0.68 (0.06)	0.69 (0.07)	0.80 (0.04)	0.80 (0.04)	671.01
O.		37		11.6 (1.6)		0.57 (0.07)		0.75 (0.04)	615.9
Mean	42	39	12.4 (0.4)	11.4 (0.4)	0.64 (0.02)	0.64 (0.02)	0.78 (0.01)	0.77 (0.01)	

Table 6: Genetic parameters of each *Ch. parallelus* population with hybrids (+) and excluding hybrids detected with the conservative approach (-)*;* N: sample size; A: mean number of alleles; H_o and H_e, observed and expected heterozygosities; N_e: mean effective population size estimate; numbers in parentheses are standard errors.

Pop.	**N +**	**N -**	**A +**	**A -**	**Ho +**	**Ho -**	**He +**	**He -**	**Ne -**
S.	39	34	16.7 (2.4)	15.0 (2.2)	0.58 (0.06)	0.59 (0.06)	0.80 (0.04)	0.81 (0.04)	4,097.7
H.	32	31	14.3 (1.9)	14.3 (1.9)	0.45 (0.07)	0.45 (0.07)	0.76 (0.06)	0.76 (0.06)	14,598.3
Z.	38	36	17.0 (2.6)	16.6 (2.5)	0.61 (0.07)	0.61 (0.07)	0.81 (0.06)	0.81 (0.06)	23,716.16
M.		36		14.3 (2.3)		0.59 (0.05)		0.8 (0.04)	8,574.6
Hd.	40	38	17.6 (2.6)	17.0 (2.6)	0.59 (0.0.4)	0.59 (0.04)	0.81 (0.04)	0.81 (0.04)	11,870.7
Ab.	32	30	14.6 (2.7)	14.0 (2.6)	0.51 (0.08)	0.51 (0.08)	0.76 (0.04)	0.77 (0.07)	4,957.3
R1	46	41	18.8 (2.4)	17.3 (2.5)	0.68 (0.06)	0.68 (0.07)	0.77 (0.06)	0.79 (0.06)	8,819.9
R2	49	41	18.0 (2.2)	16.2 (2.0)	0.64 (0.07)	0.67 (0.08)	0.77 (0.06)	0.79 (0.06)	4,283.3
F.	38	37	14.8 (2.4)	14.3 (2.5)	0.52 (0.09)	0.52 (0.09)	0.78 (0.07)	0.78 (0.07)	4,192.2
D.	39	37	16.0 (2.8)	15.1 (2.8)	0.58 (0.08)	0.58 (0.08)	0.77 (0.08)	0.77 (0.07)	9,989.2
P.	39	37	16.8 (2.6)	16.1 (2.5)	0.60 (0.08)	0.60 (0.08)	0.78 (0.08)	0.79 (0.07)	7,849.2
G.	43	41	16.7 (2.6)	16.3 (2.5)	0.55 (0.06)	0.55 (0.06)	0.77 (0.06)	0.78 (0.07)	19,020.4
B.		37		15.4 (2.8)		0.59 (0.08)		0.78 (0.07)	1,489.4
Hust		39		16.5 (2.4)		0.62 (0.05)		0.82 (0.04)	10,285.4
Mean	40	37	16.5 (0.6)	15.6 (0.6)	0.57 (0.02)	0.58 (0.02)	0.78 (0.02)	0.79 (0.02)	

We found no linkage disequilibria for any locus combination. Many loci deviated significant from HWE. F_{IS} values were generally positive, independent of whether hybrids were included in the data set or not. Even though the number of significant deviations from HWE increased in *Ch. montanus* when including hybrids, FIS values showed no significant decrease (or increase). F_{ST} values between species were significantly lower when hybrids were included than when excluding hybrids (paired t-test: t = 2.94, df = 13, P = 0.012). FST values between populations within one species increased significantly when excluding hybrids (*Ch. montanus*: paired t-test: t = 4.04, df = 13, P = 0.0014; *Ch. parallelus*: paired t-test: t = 3.84, df = 13, P = 0.002).

Correlation Analyses

We found no significant correlation between hybridization rate and any genetic parameter for *Ch. montanus* or altitude. However, for *Ch. parallelus* we found a significant positive correlation between hybridization rate and the number of alleles ($R^2 = 0.41$, $F_{1,12} = 8.3$, P = 0.014; Fig. 5). The correlation of hybridization rate and N_e was not significant, but for the populations of *Ch. montanus* there was a rather high coefficient of determination ($R^2 = 0.22$, $F_{1,10} = 2.8$, P = 0.126) with hybridization rate increasing with decreasing N_e.

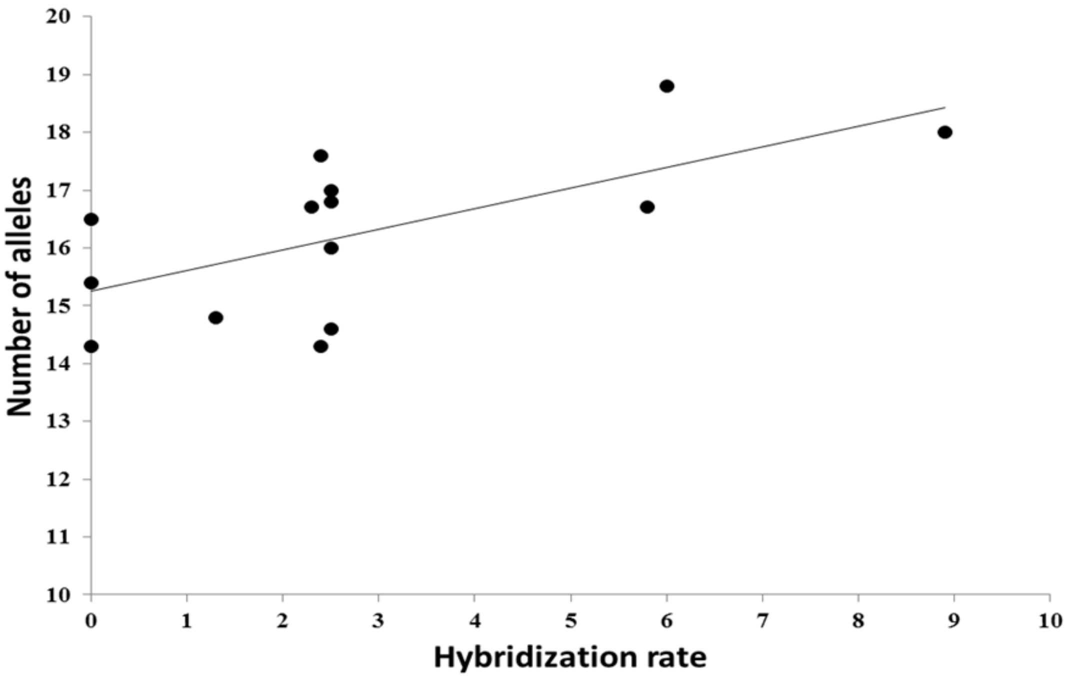

Figure 5 Correlation between hybridization rate (%) and number of alleles (A) of *Ch. parallelus* populations ($R^2 = 0.41$).

Discussion

Despite the widespread assumption that hybridization between sympatric species is rare, our results show that even species with broadly overlapping ranges hybridize in nature. Although the two grasshopper species differ in their habitat requirements and phenology, niche overlap is strong enough to allow a considerable amount of heterospecific encounters in the field (twelve of the 14 *Ch. montanus* populations were in contact with *Ch. parallelus*). Nevertheless, hybridization rate seems to be low enough to prevent a complete admixture of populations of both species. Furthermore, our lab experiment shows that hybridization risk increases with decreasing population size, i.e. increasing heterospecific encounter frequency (while increasing conspecific density did not affect the individual mating frequencies for both species). *Ch. montanus* is sensitive to droughts and habitat deterioration and has shown considerable population decline in the study area (Chapter III), whereas *Ch. parallelus* has stable (or even increasing) populations. This suggests that small *Ch. montanus* populations might face an additional risk of being genetically displaced by *Ch. parallelus*.

Evidence of hybridization

Natural hybridization between *Ch. montanus* and *Ch. parallelus* was first proposed by Chládek (Chládek 1977), who found individuals with mixed morphological characters in Slovakia. However, these morphological intermediate individuals from the Slovakian Tatry Mts. have meanwhile been described as a new species, *Chorthippus smardai* (Chládek 2014). Reynolds (1980) also recognized morphologically intermediate individuals and suspected hybridization in the wild. Other studies have shown that these two species hybridize at least under laboratory conditions with very low fitness loss of the F1 and F2 generations (Hochkirch and Lemke 2011, Köhler 2013). Our study provides the first genetic evidence that both species hybridize also in nature. In nearly all syntopic populations (except for Muhl) we identified hybrids. The hybridization rate reached a maximum of 8.9 % (but may reach up to 14.44 % when accepting the relaxed approach). The three programs varied in hybrid detection accuracy with NewHybrids performing best. Nevertheless, we recommend our approach of using all three programs as well as a prior simulation of hybrids to avoid an overestimation by a single program. With the relaxed approach we even detected hybrids in allotopic populations, which we believe to be unrealistic, even though one might argue that macropterous heterospecific individuals might occasionally immigrate. It also must be considered that STRUCTURE distinguishes neither hybrid generation nor backcrosses, which could lead to mis-assignments in some cases, leading to a more conservative estimate.

Ch. montanus and *Ch. parallelus* occur sympatrically in large parts of the Palearctic. It is thus surprising that both species regularly hybridize in nature. However, the contact between both species might be rather recent (in evolutionary terms), because *Ch. parallelus* recolonized large parts of its range during the postglacial period from Mediterranean refugia (Cooper et al. 1995, Korkmaz et al. 2014). The colonization history of *Ch. montanus* has not been reconstructed yet, but it does not occur in the Mediterranean and is generally found further north (Kleukers et al. 1997, Karjalainen 2009). This suggests that it might have colonized the temperate zone earlier or even survived here during the last glacial maximum. Hence, one may speculate that *Ch. montanus* reached its large geographic range earlier. With ongoing warming it might have become more and more restricted to higher altitudes and came in contact with *Ch. parallelus* that still expands its range (Hochkirch and Klugbist 1998).

As we found hybrids in nearly all populations and hybrids are known to have nearly no fitness loss (Köhler 2013), the question arises why the species do not mix up completely and build hybrid swarms (Schulte et al. 2012). Either the hybridization rate is still low enough to avoid complete admixture, or hybrid fitness is much lower in the field than in the lab, possibly due to mismatches of traits acting as premating barriers. Three premating barriers are usually considered important for this species pair: (1) distinct songs of both species (Faber 1929), (2) differing habitat preferences, resulting from specific drought sensitivity of the eggs (Ingrisch 1983, Köhler 2013), (3) differences in the phenology with *Ch. parallelus* becoming adult ca. one month earlier than *Ch. montanus* (Köhler 2013). It has recently been shown that the latter two aspects substantially reduce hybridization risk of both species in the field (Chapter III). Hybrids have intermediate habitat preferences and phenologies. Thus, it is unlikely that these aspects will act as efficient barriers to backcrossing of hybrids. The intermediate song of hybrids (Hochkirch and Lemke 2011) might indeed act as an efficient barrier to backcrossing hybrids, but the song differences of the parental species are much stronger and should prevent hybridization in the first place. Hence, it remains unresolved, if the lack of complete admixture is caused by such barriers or by the low hybridization rate. It is also possible that backcrosses mainly occur in one direction (with *Ch. parallelus* females), so that quasi-unidirectional gene flow occurs.

Population size and hybridization risk

Our results confirm that females of *Ch. montanus* have a much stronger preference for conspecific males than females of *Ch. parallelus* (Hochkirch and Lemke 2011). Such an asymmetric reproductive isolation seems to be common and is attributed to the ecological and reproductive differences among sexes and species (Wirtz 1999, Gröning and Hochkirch 2008, Veen et al. 2011). Differences in courtship songs of both species or even dissimilarities in pheromones (cuticular hydrocarbons) between both species could provide the underlying mechanism in the discrimination of *Ch. montanus* (Howard and Blomquist 1982, Neems and Butlin 1995). However, the role of pheromones in mate choice of these species has not been studied so far. This unidirectional barrier combined with the differences in habitat requirements and phenology may protect natural populations of *Ch. montanus* from rapid admixture with *Ch. parallelus*. This would be in line with the assumption that multiple barriers cause restriction of gene flow between closely related species (Coyne and Orr 2004). We assume that the asymmetry in female choosiness has evolved as a consequence of the different encounter probabilities caused by their differing ecology and distribution. While most *Ch. montanus* populations occur in syntopy with *Ch. parallelus*, the latter species has a very wide distribution and occurs only rarely syntopically with *Ch. montanus*. Therefore, selection pressure on reproductive barriers affects a higher proportion of *Ch. montanus* females, but only a very small proportion of *Ch. parallelus* females (Gwynne and Morris 1986, Wirtz 1999). However, it is also possible that the lower choosiness is caused by the age of females. As *Ch. parallelus* becomes adult earlier, they might have a reduced choosiness (i.e. higher receptivity) than those of *Ch. montanus*, which are still younger.

The records of hybrids from natural populations show that hybridization is not an artifact produced by laboratory conditions. It confirms that interspecific mating occurs regularly in the wild despite the existence of ecological, phenological and ethological barriers. Mate choice strongly depends on the encounter rate of potential mates and the costs and benefits of mate choice (Milinski and Bakker 1992, Willis et al. 2011). Low encounter rates with conspecific mates increase the costs of mate searching and reduce choosiness (Milinski and Bakker 1992, Willis et al. 2011). Our mate choice experiment demonstrates a decreasing frequency of conspecific matings and an increasing number of cross-matings with increasing heterospecific density for females of both species, but females of *Ch. montanus* only chose heterospecific males at the highest density of heterospecifics (1:4). This suggests that even the bioacoustic differences of both species are not sufficient to ensure a “correct” mate choice. If the direct contact of individuals is more important for mate finding than the song, the encounter probabilities might determine hybridization risk (Gröning and Hochkirch 2008). Songs may only be important at low densities to find corresponding mates (Butlin and Hewitt 1986, Ingrisch and Köhler 1998).

The results of our lab experiment suggest that demography might be a major driver of hybridization in wild syntopic populations. We suppose that in large populations of *Ch. montanus* hybridization is rare and restricted to the periphery of the habitat, which might lead to the formation of a mosaic hybrid zone, but not to genetic displacement (Köhler 2013). If a *Ch. montanus* population decreases in size and abundances become more and more skewed towards *Ch. parallelus*, the reproductive barrier might weaken as has been shown for other rare species (Mayr 1963, Wirtz 1999, DeWoody et al. 2010, Willis et al. 2011). Hence, a population decline caused by land use change (abandonment), drainage or climate change (Weyer et al. 2012, Chunco 2014) might lead to a vortex effect, increasing the strength of other threats such as hybridization. In fact, we monitored the population dynamics of the R1 and R2 populations from 2010 to 2014 (Chapter III) and found that *Ch. montanus* declined by 90.3% on R1 and by 49.6% on R2 during this period. We assume that the decline was mainly driven by weather conditions (there were severe droughts in spring and autumn 2011, which might have caused the severe population decline of R1 by 87% until 2012) or ongoing accumulation of grass debris at the sites due to abandonment. However, this population decline might increase the risk of future hybridization or even might be increasingly caused by hybridization itself.

Genetic diversity and hybridization

It is well known and consistent that hybridization increases genetic diversity within a population (Schulte et al. 2012). Population augmentation is therefore sometimes used in conservation management to avoid inbreeding depression at low population size (Johnson et al. 2010). As long as *Ch. montanus* populations remain large and stable, a leaky reproductive isolation could increase genetic variability (Hedrick 1995, Arnold et al. 2008, Abbott et al. 2013). However, hybridization can also lead to a near-complete genetic displacement of a species. Hedrick (2009) compared introgression of red wolf populations from coyote populations with Wright’s continent-island model (Wright 1931), i.e. with unidirectional gene flow. This is probably an oversimplification as gene flow would necessarily affect both populations and thus would follow Wright’s general island model, i.e. gene flow in both directions. This means that the larger gene pool of *Ch. parallelus* will displace the gene pool of *Ch. montanus* until an equilibrium is reached. A new, completely admixed population will thus conserve some *Ch. montanus* alleles at a very low frequency (reinforced by heterosis; (Fitzpatrick and Shaffer 2007)). This is similar to Neanderthal alleles being still present in the human gene pool (Sankararaman et al. 2014), but the genetic integrity of the *Ch. montanus* population would be lost (DeWoody et al. 2010). By contrast, the genetic diversity of large populations of *Ch. parallelus* increases with occasional hybridization. It remains unknown, whether this may represent an advantage (higher adaptability) or a risk (genetic incompatibilities) in the long term.

Conclusions

Our results support the hypothesis that hybridization between the sympatric sister species *Ch. montanus* and *Ch. parallelus* also occurs in the wild. We assume that naturally hybridization mainly takes place in ecotones between wetlands and drier habitat types, where both species come into contact. As cross-mating probability increased in the lab with decreasing relative frequency of conspecific mates, we conclude that the encounter rate is a major driver of hybridization. Population decline caused by stochastic and environmental fluctuation will thus increase the probability of hybridization as an additional threat. Habitat restoration and wetland management are therefore important tools to save this species from such vortex situations.

Acknowledgements

We acknowledge the “Struktur- and Genehmigungsdirektion Nord” for permitting the collection of samples and access to nature reserves. We also acknowledge Jasmin Weinberger and Marielle Mayer for sampling as well as Elena Kaut and Bernd Schneider for laboratory assistance. The study was funded by the German Research Foundation (DFG, GRK 1319) in the interdisciplinary graduate school ‘Cooperation of Science and Jurisprudence in Improving Development and Use of Standards for Environmental Protection – Strategies for Risk Assessment and Management’.

Availability of Supporting Data

The microsatellite data of this paper are deposited at Dryad data repository (doi:10.5061/dryad.1dd0m).

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Chapter III

Extreme climatic events cause rapid population declines of a rare wetland species and increase hybridization risk with a habitat generalist

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Unpublished manuscript

Abstract

Climate change and climatic extremes may affect species directly or indirectly. While direct climatic effects have been intensively studied, indirect effects, such as increasing hybridization risk, are poorly understood. We studied the impact of climate on population dynamics of a rare habitat specialist, *Chorthippus montanus*, and on hybridization with a sympatric habitat generalist, *Chorthippus parallelus*. We conducted mark-recapture studies on two sites over five years and tested for correlations with climate data and genotyped 702 individuals of two *Ch. montanus* generations to detect hybrids. We first tested the performance of three programs (STRUCTURE, NewHybrids, adegenet) and then accepted only hybrids detected by the two best performing programs. The highest hybridization rate (19.6%) was found in the population with lowest population size. Our results reveal that climatic extremes trigger strong population declines in the habitat specialist. This in turn, leads to increasing heterospecific encounter and may thus increase hybridization risk. Our data also show that spatiotemporal niche overlap decreases heterospecific encounter probabilities to 4.2-7.6 % compared to 20-28 % and 11-19 % calculated alone from phenology or spatial overlap, respectively. This illustrates that the combination of spatial and temporal segregation provides an effective barrier, although this function decreases with decreasing population size. Hybrids were located mainly at the edge of the specialists' occupied habitat in areas with intermediate soil moisture conditions compared to the parental species. This confirms that climatic extremes threaten rare species both directly by reducing reproductive success and indirectly by increasing hybridization risk.

Keywords: climate change, extreme climatic events, genetic displacement, microsatellite, reproductive barriers, sympatric species

Introduction

It is widely agreed that climate change can have dramatic consequences for ecosystems, biodiversity and species' distributions. Habitat modifications, range shifts of species as well as altered species interactions are considered to be the most important consequences (Parmesan et al. 1999, Visser and Both 2005, Parmesan 2006, Chunco 2014, Sánchez-Guillén et al. 2014). The extent and rate of responses to climate change vary strongly depending on the species involved and their physiological tolerances, dispersal ability and life history strategies (Parmesan 2006, Chunco 2014, Sánchez-Guillén et al. 2014). Short-lived species generally seem to respond faster than long-lived species, however, even closely related species could differ in their response as a result of their differing ecological specialization (Bale et al. 2002, Tingley et al. 2009, Angert et al. 2011, Chunco 2014, Sánchez-Guillén et al. 2014). Habitat generalists are predicted to be less sensitive to climate change than specialists due to their wider range of tolerances to environmental changes (Gilchrist 2000). Moreover, the effects of climate change are unlikely to proceed in a constant pattern. It is more likely that climatic extremes, such as droughts and strong rainfall will lead to sudden changes in population dynamics (Hochkirch and Damerau 2009).

The effects of climate change on reproductive interactions between closely related species are little understood (Garroway et al. 2010, Zamundio and Harrison 2010, Hochkirch and Lemke 2011, Chunco 2014). If the species' responses to climatic changes are asynchronous, equilibria are likely to become disrupted. For hybridizing species, climate change may alter the function of reproductive barriers or the equilibrium of their interactions (Engler et al. 2013). An asynchronic range shift for example could lead to a secondary contact between formerly allopatric species and form novel sympatric populations, which may result in new interspecific interactions and hybridization processes (Gröning and Hochkirch 2008, Chunco 2014, Sánchez-Guillén et al. 2014). Furthermore, such an asynchrony may alter the dynamics of sympatric populations by increasing their degree of sympatry (Sánchez-Guillén et al. 2013, Sánchez-Guillén et al. 2014). Although sympatric species may have evolved reproductive barriers as a result of negative selection to hybridization (Rundle and Schluter 1998, Hochkirch and Lemke 2011), environmental change could lead to a collapse of such reproductive barriers (Seehausen 2006, Crispo et al. 2011, Hasselman et al. 2014). Besides the effects of shifting ranges, climate change may also influence the phenology of species and thus the temporal reproductive isolation of closely related species (Cleland et al. 2007, Parmesan 2007, Chunco 2014).

Additionally, hybrid fitness could be affected by climate change (Chunco 2014) and hybrids may sometimes have a better adaptation to the novel environmental conditions than the parental species (Rieseberg 1997, Arnold et al. 2008).

Climatic effects on hybridization interactions are particularly interesting, as they could have severe evolutionary consequences, such as speciation (Abbott et al. 2013, Hochkirch 2013), but are also of conservation concern because of genetic displacement processes and outbreeding depression (Rhymer and Simberloff 1996). The consequences of hybridization can vary considerably depending on the species involved. Hybridization could lead to new adaptations in a changing environment, if hybrids are fertile and niches are available (Mallet 2005) or it may enhance genetic diversity and prevent small populations from inbreeding depression (Mallet 2005, Arnold et al. 2008, Schulte et al. 2012). These positive effects of hybridization were generally described for natural hybridization processes whereas anthropogenic hybridization is more often considered a threat, particularly to rare species and small populations (Rhymer and Simberloff 1996, Seehausen et al. 2008). Anthropogenic hybridization is mainly discussed in the context of invasions of non-native or domesticated species or in ex situ conservation programs (Huxel 1999, Allendorf et al. 2001, Randi 2008, Witzenberger and Hochkirch 2014), whereas indirect facilitation of hybridization via habitat loss or climate change has received only little attention (Seehausen et al. 2008, Kelly et al. 2010, Sánchez-Guillén et al. 2013, Chunco 2014). Anthropogenic hybridization may lead to a population collapse due to genetic displacement which may result in local extinction of populations or even the complete extinction of a species (Rhymer and Simberloff 1996, Seehausen et al. 2008).

The two grasshopper species *Chorthippus montanus* (Charpentier, 1825) and *Chorthippus parallelus* (Zetterstedt, 1821) represent an interesting study system concerning the potential effects of climate on population decline and hybridization risk. Both species occur sympatrically across large parts of Eurasia (Fig. S1), but *Ch. montanus* is a habitat specialist adapted to permanently moist habitats, which are naturally fragmented, whereas *Ch. parallelus* is a widely distributed habitat generalist occurring in a variety of non-arid grasslands (Detzel 1998). Both species are closely related and morphologically very similar, but have distinctive songs (Reynolds 1980). Hybridization between both species in the field has been first assumed by Reynolds (1980) and confirmed in a previous study (Chapter II). Laboratory experiments have shown that hybrids are fertile at least until the third generation (Reynolds 1980, Bauer and von Helversen 1987, Köhler 2013). Hybrids are morphologically either intermediate or similar to *Ch. parallelus* and perform intermediate songs (Hochkirch and Lemke 2011, Köhler 2013). Even though *Ch. montanus* females strongly prefer conspecific males as mates, mate choice is frequency dependent and increasing heterospecific frequency increases the risk of hybridization (Chapter II).

During the last two decades, *Ch. montanus* has disappeared from sites < 400 m in the Hunsrueck Mountains (Rhineland-Palatinate, Germany; Weyer et al. 2012), possibly as an effect of climatic extremes, such as the summer heatwave in 2003 or spring drought in 2007 (Figs. S2-S4). Furthermore, we found single intermediate morphotypes at localities with former occurrence of *Ch. montanus*. Therefore, we hypothesized that hybridization with *Ch. parallelus* poses an additional threat to small populations. In order to test the hypothesis that climatic fluctuations influence the population trend of *Ch. montanus*, we studied the dynamics of two populations from 2010 to 2014 using a mark-recapture study. We then tested for correlations with climatic parameters, including climatic extremes, such as the drought in April 2011. In order to detect hybrids we genotyped all *Ch. montanus* specimens captured in 2012 and 2013. We further hypothesized that the low spatio-temporal overlap between both species is the major premating barrier and more effective than the spatial or temporal overlap alone. Therefore, we conducted a mark-recapture study also for the population of *Ch. parallelus* on one of the sites from 2011 to 2013 and used a GIS analysis to calculate the spatio-temporal population overlap. Moreover, we tested if hybrids were mainly located in areas with high heterospecific encounter probabilities at the edge of the *Ch. montanus* distribution. Finally, we hypothesized that the species strongly differ in their soil moisture preferences and that hybrids occupy zones of intermediate moisture.

Materials & Methods

Study species

Chorthippus montanus is a univoltine, hygrophilous grasshopper species, which occurs in moist habitat types such as permanently moist grasslands, water meadows, marshes, and peat bogs due to the high water requirements of the eggs (Froehlich, 1994; Ingrisch, 1983b; Kleukers *et al.*, 1997). Ongoing habitat deterioration caused by land use change such as abandonment and drainage represent the main threats for *Ch. montanus* populations in the study region (Weyer *et al.*, 2012). The species is classified as near threatened in Germany (Maas *et al.*, 2002) and red-listed in several European countries (Decleer *et al.*, 2000; Kleukers *et al.*, 1997; Thorens, Nadig, 1997). The species is flightless and its mobility is low (Weyer *et al.*, 2012). Nymphs hatch in May and the adults are found from July to November. *Chorthippus parallelus* is a common univoltine grasshopper species (Kleukers *et al.*, 1997). The phenology of both species is slightly shifted, with the nymphs of *Ch. parallelus* hatching earlier than those of *Ch. montanus* and becoming adult ca. one month earlier (Hochkirch, Lemke, 2011; Ingrisch, Köhler, 1998).

Study site

Our previous study on the hybridization rate between both species has shown that the populations Reinsfeld 1 (R1: N49°40'26.674"; E6°52'59.516") and Reinsfeld 2 (R2: N49°41'11.504"; E6°53'58.412"; Fig. S1) had the highest hybridization rate (Chapter II). Although both study sites are located in close vicinity (linear distance 1.7 km) and at similar altitudes (R1: 480 m; R2: 520 m asl), both *Ch. montanus* populations showed a striking difference in phenology in 2010. The population maximum on R1 was recorded on 10 August, whereas on R2 (as well as on two other sites) it was found on 1 September. Based on these phenological differences and the potential consequence for phenological overlap with *Ch. parallelus* these sites were chosen for our studies of the spatio-temporal population dynamics and occurrence of hybrids. Both study sites represent isolated moist meadows. The *Ch. montanus* populations are strongly isolated as well with no gene flow to other populations in the vicinity (Weyer et al. unpubl.). The habitat size of R1 is smaller (9,353 m²) than of R2 (11,747 m²). Both *Ch. montanus* populations were surrounded by large (and continuous) *Ch. parallelus* populations.

Data Collection

In 2010, we conducted a first sampling of ca. 40 individuals of each species on both study sites for a preliminary genetic study, by collecting single hind legs of each individual (Chapter II). To study the population dynamics of *Ch. montanus*, a mark-recapture study was performed during five years (2010-2014) for both populations. From 2011 to 2013, the *Ch. parallelus* population on R2 was also studied by mark-recapture to investigate the spatial and temporal population overlap of both species. During each visit, the study site was completely combed (usually by two persons) and each individual was caught with a net. Each specimen was individually marked with a non-toxic permanent paint marker (Edding 780) using a modified 1-2-4-7 method (Weyer *et al.*, 2012) and was subsequently released at the same position. Additionally, the following parameters were recorded: individual number, date, sex, number of hind legs and geographic coordinates of the capture position of each individual. To analyze the spatial distribution of both species and hybrids, the geographic coordinates were determined with the Trimble® GeoXT™ GPS device (GeoExplorer® 2008 series) and a Trimble® GeoBeacon™, a receiver for high quality real-time differential corrections. In 2012 and 2013, a tarsus of one hind femur of each individual was sampled for genetic analyses and hybrid detection (N_{total}: 702). The mark-recapture study was performed from end of July to end of October. The sites were visited every 2-5 days depending on the weather conditions and the study was stopped when the number of individuals dropped below 3 individuals.

Mark-recapture analysis

The program MARK 4.3 (White, Burnham, 1999) was used to estimate population sizes of both species and sites for each year to analyze population trends. Furthermore, daily population sizes were calculated for each species to test the temporal overlap. The module POPAN was used to perform Jolly-Seber calculations allowing the calculation of population sizes of open populations with differing death and recruitment rates over life time. Three different parameters are estimated: the daily survival probability Phi_i including death and emigration, the daily recapture probability p_i and the daily immigration probability $pent_i$ regarding immigration and birth. With these parameters and the recapture data the total daily population size N_i and the total population size N are estimated (Fric *et al.*, 2009). In order to test the quality of our data we first ran the full model (*Phi*(g*t)*p*(g*t)*pent*(g*t)N(g); g=sex, t=time) and performed a goodness of fit test subsequently. Afterwards predefined and simplified models were calculated to reduce the number of parameters included. For each of the three parameters, we modified the explanatory variable and combined these in all possible combinations. We tested the interaction term of sex and time (g*t), the addition of sex and time (g+t) as well as sex (g) and time (t) independently or as constant parameters (.). For Phi_i we also tested the addition of sex and a linear trend for temporal effects (g+T), which often applies to grasshopper populations as the survival probability decreases with time over the capture season. The best model for population size was chosen using the Akaike Information Criterion (AIC).

Weather Data

In order to investigate the impact of weather on population dynamics of *Ch. montanus*, we analyzed the daily weather data for the years 2010-2014 from the weather station "Trier

Petrisberg" of the German Meteorological Service (DWD). The data was divided in three different phases (phase1 = reproductive phase of the previous year, August-October; phase2 = egg phase, November-April; phase3 = nymphal development, May-July). We tested the impact of the weather variables on the yearly population change using hierarchical partitioning (package hier.part in R), to detect the average independent contribution of each weather variable (Chevan, Sutherland, 1991). The following weather variables were selected as they are likely to be important considering the ecology of *Ch. montanus*: days without rainfall, longest arid period, precipitation, air temperature (average, maximum, minimum at ground level), cloudage, wind velocity, snow (only in the egg phase) and sunshine hours on population change. Afterwards, the correlation of population changes with the three strongest explanatory variables predicted by hierarchical partitioning was tested in linear regression models. To illustrate the multidimensional correlations between weather variables during the three different phases and their correlation with population changes, we performed Principal Component Analyses (PCA) in R using the vegan 2.0-10 package (Oksanen *et al.*, 2013). Each factor was scaled by its proportional eigenvalue due to the strong variability of the scales of our data. We used environmental fitting (env.fit) with 1,000 permutations to test the correlations of the population changes on each site with the PCA functions. This method tests if random permutations of the variables yield a higher degree of fit than the true variables and produces an R^2 measure and significance values. All tests were carried out in R 3.0.2 (R Development Core Team, 2014).

Genetic analyses

In order to detect hybrids and study the genetic consequences of population size changes, we used a microsatellite study. DNA was extracted from the tarsus of the hind leg using the DNeasy Blood & Tissue Kit (Qiagen). All individuals (N_{total}: 702) were genotyped at ten polymorphic microsatellite loci. Four microsatellite markers were designed for *Ch. montanus* prior to this study (CM5, CM19; CM 33; CM 37; Table 3 Chapter II) and six were developed for *Ch. parallelus* (BF1, BD5, BH5, BD7, BF9, CD6; Molecular Ecology Resources Primer Development Consortium, Abercrombie *et al.*, 2009).

The Qiagen Multiplex Mastermix was used in multiplexed PCR protocols for a combination of two to four loci with the following annealing temperatures (BF1, BH5, CD6, CM37: 54°C; BD5, CM5: 48°C; CM33, CM19: 51°C; BD7, BF9: 58°C). We filled PCR tubes with 10 µl reaction mixes (5.5 µl MultiplexMasterMix, 2 µl water, 1.4 µl genomic DNA (2-10 ng), 1.1 µl primer mix (1 µM / primer)). Amplification was carried out in a Multigene Gradient Thermal Cycler (Labnet) with the following PCR conditions: Initialization: 94°C/10 min; Denaturation: 94°C/45 sec; Annealing: see primer/45 sec; Extension: 72°C/45 sec; Final Extension: 72°C/30 min; 37 cycles. Each forward primer was labeled with a fluorescent dye (FAM, HEX or TAMRA) at the 5`-end. Fragment lengths of PCR products were determined on a MEGABACE 1000 automated sequencer (GE Healthcare) and scored with Fragment Profiler 1.2 (Amersham Biosciences).

Simulating and detecting hybrids

In order to detect hybrids in the data set, we simulated hybrids in HYBRIDLAB 1.1 (Nielsen *et al.*, 2006). This simulation was based upon a subset of around 40 purebred individuals of each parental species and each study site identified during a previous study (Chapter II). Four classes of hybrids (F1, F2 and backcrosses with both species) with 50 individuals of each class were simulated using HYBRIDLAB 1.1 (Nielsen *et al.*, 2006). We then tested the performance of hybrid detection in this simulated data set with three programs, STRUCTURE 2.3.4, NewHybrids (which represent a Bayesian approach) and the R package adegenet 1.4-1 (which uses a discriminant analysis of principal components) (Anderson, Thompson, 2002; Jombart, 2008; Pritchard *et al.*, 2000). For STRUCTURE we first determined the optimal threshold (q-value) to minimize the number of mis-assignments based upon a larger simulated data set of 200 individuals per hybrid class as well as 119 purebred *Ch. montanus* and 118 purebred *Ch. parallelus* from a preceding study (Chapter II). The STRUCTURE analysis was run with the admixture model, a burn-in of 10^4 simulations followed by 10^5 Markov chain Monte Carlo (MCMC) simulations and a K of two with ten iterations. The optimal threshold was detected at $q = 0.91$, i.e. hybrid assignment between 0.09 and 0.91. However, even with this optimal q value the percentage of mis-assignments in STRUCTURE was substantially higher (15.9%) than in adegenet (11.0 %) and NewHybrids (12.0 %; Table S1). Therefore, we only used the latter two programs for hybrid assignment.

The program NewHybrids assigns individuals to different hybrid classes (i.e. F1, F2 and backcrosses; Anderson, Thompson, 2002). For our analysis we just distinguished between the two purebred parental species and a category “hybrid” (including F1, F2 and backcrosses). Posterior distributions were evaluated after 10^5 iterations of the MCMC and a burn-in period of 10^4 iterations. Adegenet 1.4-1 is using a discriminant analysis of principal components (DAPC) and a posthoc classification of individuals to one of three classes (both purebred species and "hybrid"). As both programs overestimated the number of hybrids, we only defined those individuals as hybrids, which were detected by both programs. The hybridization rate refers to the number of hybrids in relation to the total number of genetically sampled *Ch. montanus* individuals.

Spatial Analyses

Density distribution and encounter probabilities

In order to calculate the spatial and spatio-temporal encounter probabilities as a proxy of hybridization risk, we used the GPS data from the mark-recapture studies in 2012-2013 for further analysis in ArcGIS 10.1 (ESRI, 2011). For *Ch. montanus* we analyzed the spatial density distribution at both sites and years to calculate its density in 1 m² grid cells. For *Ch. parallelus* we calculated the same for the area covering the complete *Ch. montanus* distribution to test the overlap of both species in this area. The calculation was done (1) for the complete study period (i.e. pure spatial overlap) and (2) for seven periods á two weeks from end of July to end of October (i.e. spatio-temporal overlap) using the Spatial Analyst tool *Kernel Density* (settings: cell size=1; search radius=1; Unit= m²). To receive values per m², we created a \`distribution´ grid shapefile (1x1 m²) across the corresponding site and transformed the density grid into a point shapefile using the conversion tool *Grid to Point.* Afterwards we merged both grids using the function *Join*. Using these data, we calculated the abundance of both species per m² for each time period and defined the relative frequency of *Ch. parallelus* in cells of *Ch. montanus* as heterospecific encounter probability in that grid cell ($EP_i = N_{para(i)}/(N_{mont(i)}+N_{para(i)})$, with EP = heterospecific encounter probability, N = abundance, i = grid cell). We then weighted each grid cell with the relative number of *Ch. montanus* individuals occurring within it to obtain the overall heterospecific encounter probability for the complete grid in that time period ($EP_t = (\Sigma(EP_i * N_{mont(i)}))/N_{mont(t)}$, t = total). To compare the heterospecific encounter probabilities in areas of the next generation hybrids with those in areas of the next generation purebred *Ch. montanus,* we calculated the mean heterospecific encounter probabilities of the preceding year within a radius of five meters of each individual using the Geoprocessing Tools *Buffer* and *Intersect*. A two-way ANOVA was used to test for significant differences in the encounter probabilities between purebred *Ch. montanus* and hybrids as well as among years. The response variable was Box-Cox-transformed using the MASS library for R to infer the optimal lambda to achieve an optimal data distribution for ANOVAS (Venables, 2002).

For creating GIS maps, the locality of each recaptured individual was averaged using the Data Management Tool *Multi Convex Polygon* with the geometry Convex_Hull (no angle over 180°) to avoid pseudoreplication. Afterwards the Spatial Analyst Tool *Kernel Density* with the settings: cell size=1; search radius=5 and unit= m^2, was used to create a density map of *Ch. montanus* for each site and year. A search radius of 5 m^2 was chosen, because the species has generally a very low mobility (Weyer *et al.*, 2012).

Soil moisture

In order to analyze the soil moisture preferences of both species and the hybrids, the soil moisture of each capture position was recorded using a UMS Infield 7 tensiometer with a Theta probe (type ML2x) during the mark-recapture study. A two-way ANOVA was used to test for significant differences in the soil moisture of capture position between species (*Ch. montanus, Ch. parallelus* or hybrid), site and year. To visualize the soil moisture variation on each site and to identify possible ecological boundaries we created a soil moisture map in ArcGIS 10.1 (ESRI, 2011). A 5 x 5 meter grid was established on both sites marked with bamboo sticks and soil moisture was measured in 2012, 2013 and 2014. During each measurement, we measured the soil moisture at each grid node three times using the tensiometer UMS Infield 7 with Theta probe (type ML2x) and calculated a mean. The GPS coordinates were recorded using the Trimble® GeoXT™ (GeoExplorer® 2008 series) and the Trimble® GeoBeacon™. Soil moisture fluctuates strongly depending on the current waterlogging. However, our intention was to obtain data on relative soil moisture variation across the study site. Therefore, it was measured during relatively dry periods. The mean soil moisture across study years was finally calculated for each grid node to infer a soil moisture map using the Spatial Analyst Tool *Interpolation* (Kriging) with the settings Krigingmethod = spherical and search radius = 12 points.

Results

Population size and the correlation with weather data

Both *Ch. montanus* populations strongly declined over the years. From 2010 to 2014 the R1 population of *Ch. montanus* decreased by 90.3% and the R2 population by 49.6% (Fig. 1). The *Ch. parallelus* population decreased from 2011 to 2013 by 21.3% (Table 1). The strongest decline was found for both *Ch. montanus* populations from 2011 to 2012 with a decline of 72% for R1 and 35% for R2.

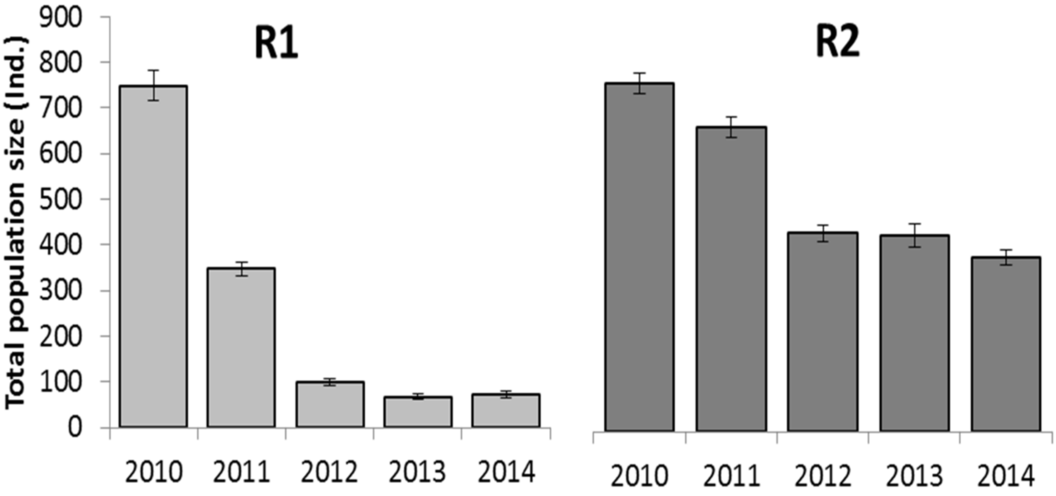

Figure 1 Estimated total population size of *Ch. montanus* on the two sites Reinsfeld1 and Reinsfeld2 using the program MARK. Error bars are standard errors.

The hierarchical partitioning analysis revealed that the weather parameters explaining population changes varied among the three phases. During the reproductive phase of the preceding year, the length of the arid period had the highest impact on population change and showed a significant negative correlation ($R^2 = 0.5$, $F_{1,6} = 6.1$, $p < 0.05$). Wind velocity and precipitation were the next important variables identified by hierarchical partitioning, but they were not significantly correlated with population change. During the egg phase, days without rain and length of the arid period had the highest impact on population change. The regression analysis showed a significant negative correlation for days without rain ($R^2 = 0.5$, $F_{1,6} = 7.3$, $p < 0.05$) and a similar trend for the length of the arid period ($R^2 = 0.48$, $F_{1,6} = 5.4$, $p = 0.058$). During the nymphal phase, population change was significantly negatively correlated with cloudage ($R^2 = 0.5$, $F_{1,6} = 6.4$, $p < 0.05$) and showed a trend for a positive correlation with days without rain ($R^2 = 0.43$, $F_{1,6} = 4.6$, $p = 0.076$).

The PCAs for the weather data during the three different phases were rather different. The two first principal components explained 88 % (reproductive phase, PC1: 68.4, PC2: 19.9 %), 83 % (egg phase, PC1: 52.5, PC2: 30.4 %) and 88% (nymphal phase, PC1: 58.3, PC2: 31 %) of the variance (Figs. 2A-C). During the reproductive phase, the first function was mainly explained by days without rain (0.74) and cloudage (-0.70), while the second function was explained by minimum air temperature (0.71). During the egg phase, the first function was mainly explained by mean air temperature (0.74), while the second axis was explained by days without rain (0.68) and length of the arid period (0.65). During the nymphal phase, the first function was mainly explained by sunshine hours

(-0.72) and days without rain (-0.72), while the second axis was explained by cloudage (-0.61) and length of the arid period (0.61). Only during the nymphal phase, population change of the R1 population was significantly correlated with the principle components (environmental fitting: p = 0.03, PC1 = 0.72, PC2 = -0.69), whereas no significant correlation was found for R2.

Table 1: Estimation of the total population size of *Ch. montanus* and *Ch. parallelus* on the sites Reinsfeld 1 (R1) and Reinsfeld 2 (R2) using the best models calculated with the program MARK. For *Ch. parallelus* the estimated population size for the overlapping area of both species on R2 (OA) is also shown. Population change is the percentage change of population size from one year to the next. Phi: daily survival probability; p: daily recapture probability; pent: daily immigration probability; N: population size. The parameters g: sex and t: time may be constant (.), independently, in interaction (g*t) or in addition (g+t). T represents a linear trend for temporal effects.

Species & Site	Year	Best Model (MARK)	Total Population size	Population change (%)	Date of max. daily population size
Ch. montanus	2010	Phi(g+T)p(g)pent(g*t)N(.)	749 ± 34		10 Aug
R1	2011	Phi(g+T)p(g+t)pent(t)N(g)	347 ± 14	-53.8	26 Aug
	2012	Phi(.)p(g)pent(t)N(.)	99 ± 8	-71.5	27 Aug
	2013	Phi(g+T)p(g)pent(g+t)N(g)	67 ± 6	-32.3	22 Sep
	2014	Phi(g+T)p(g)pent(g+t)N(g)	73 ± 7	9	28 Jul
Ch. montanus	2010	Phi(g+T)p(g+t)pent(t)N(g)	755 ± 23		01 Sep
R2	2011	Phi(g+T)p(g+t)pent(t)N(g)	661 ± 22	-12.5	01 Sep
	2012	Phi(g+T)p(g+t)pent(t)N(.)	431 ± 18	-34.8	31 Aug
	2013	Phi(g+T)p(g)pent(g*t)N(g)	426 ± 26	-1.2	13 Sep
	2014	Phi(g+T)p(t)pent(g*t)N(g)	378 ± 15	-11.3	01 Sep
Ch. parallelus	2011	Phi(g+T)p(t)pent(g+t)N(g)	1517 ± 69		04 Jul
R2	2012	Phi(g+T)p(g+t)pent(g+t)N(g)	1435 ± 167	-5.4	31 Jul
	2013	Phi(g+T)p(t)pent(g*t)N(g)	1194 ± 91	-16.8	31 Jul
Ch. parallelus	2011	Phi(g+T)p(t)pent(g+t)N(g)	416 ± 39		26 Jun
OA	2012	Phi(g+T)p(g+t)pent(g+t)N(g)	384 ± 123	-7.7	26 Jul
	2013	Phi(g+T)p(t)pent(g*t)N(g)	289 ± 45	-24.7	29 Aug

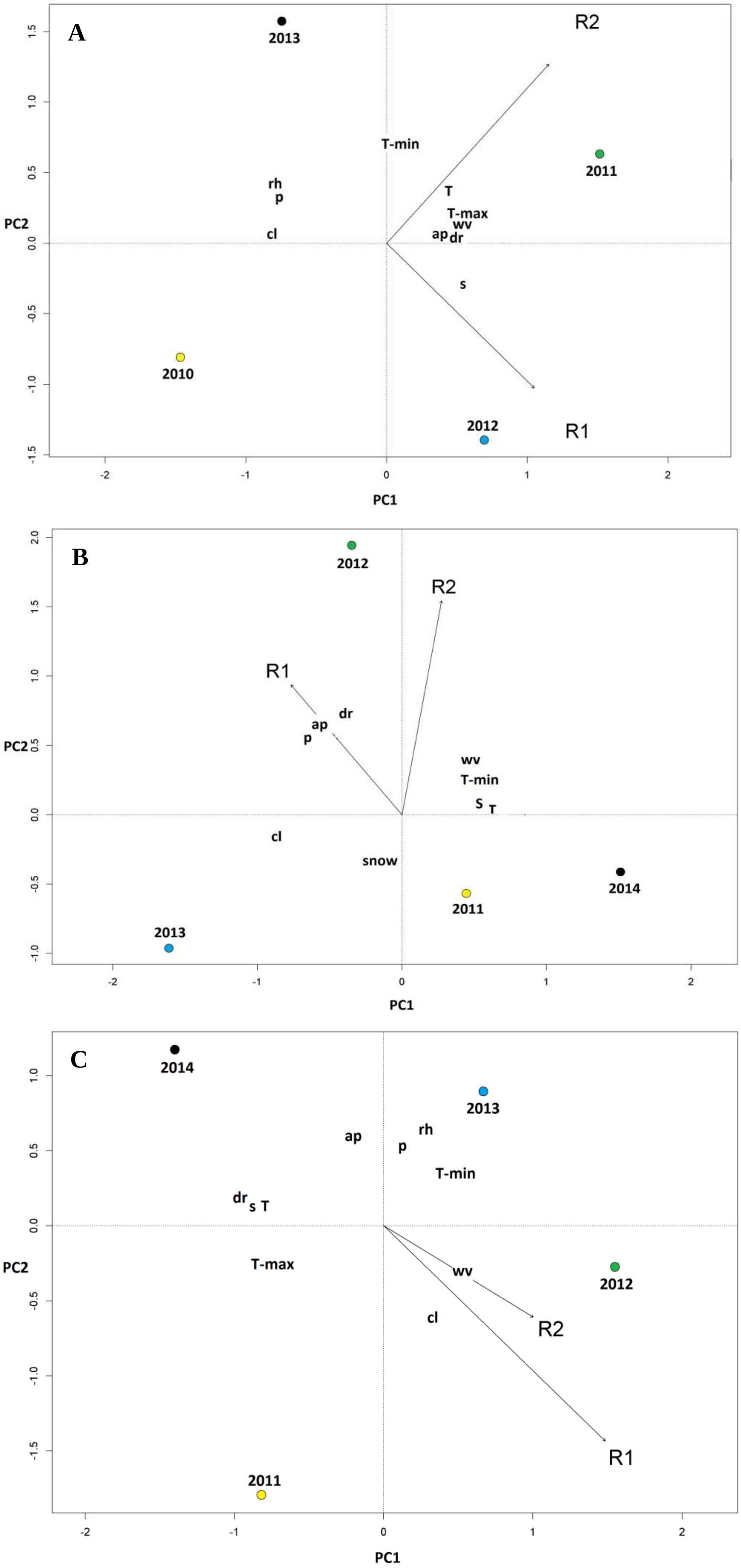

Figure 2 Plot of the first two functions of the Principal Component Analyses (PCA) on the variables averaged air temperature (T), minimal air temperature (T-min), maximal air temperature (T-max), days without rainfall (dr), sunshine hours (s), wind velocity (wv), cloudage (cl), length of the arid period (ap), precipitation (p) and relative air humidity (rh) for the reproductive phase in the previous year (a), the egg phase (b) and the nymphal phase (c), explaining 88% (A; PC1: 68.4 %, PC2: 19.9 %), 83 % (B; PC1: 52.5 %, PC2: 30.1 %) and 89 % (C; PC1: 58.3 %, PC2: 31 %) of the total variance. Each point represents one year and the colour represents the corresponding development period (yellow: 2010-2011; green: 2011-2012; blue: 2012-2013; black: 2013-2014). Arrows show the correlation of population shift of R1 and R2 using environmental fitting.

Hybridization rate

The hybridization rate of the *Ch. montanus* population on R1 was 15.6 % (10/64) in 2012 and 19.6 % (11/56) in 2013. On R2 the hybridization rate was 7.4 % (23/298) in 2012 and 6.0 % (17/284) in 2013. If the analysis was based solely on adegenet, the hybridization rate on R1 would be 39.1 % for 2012 and 42.9 % for 2013, whereas on R2 the values would be 11.4 % and 8.8 %. For NewHybrids, the hybridization rate on R1 would be 29.7 % in 2012 and 32.1 % in 2013 and on R2 8.4 % in 2012 and 7.4 % in 2013. We suspect that the larger variation of estimates on R1 is a consequence of the small population size on this site and a potentially higher number of backcrosses. In fact, the performance tests showed that mis-assignments of both programs were mainly either backcrosses assigned as purebred individuals or *vice versa*.

Species distribution and overlap

Ch. parallelus reached its maximum daily population size between the beginning (2011) and end of July (Table 2). The date of maximum daily population size of both *Ch. montanus* populations varied between the sites. While on R2 the maximum daily population size was rather constant at the end of August or beginning of September, it fluctuated strongly between the years on R1 between end of July (2014) and end of September (2013; Table 2, Fig. 3).

The GIS analyses showed that the occupied area of *Ch. montanus* on R1 had a size of 2,986 m² in 2011 which decreased during the following years (2012: 581 m², 2013: 560 m²). On R2, *Ch. montanus* had an occupied area of 1,186 m² in 2011 (2012: 713 m², 2013:720 m²), of which 585 m² (49.3 %; 2012: 327 m², 45.9 %; 2013: 326 m², 45.3 %) were also occupied by *Ch. parallelus*.

The ratio of the daily population sizes of both species within the overlapping zone of *Ch. montanus* and *Ch. parallelus* (R2) changed at the end of June (2011), the mid of August (2012) or the end of July (2013). At the beginning of the season *Ch. parallelus* was generally more frequent in the overlapping zone than *Ch. montanus,* afterwards *Ch. montanus* became more frequent (Fig. S5). The heterospecific encounter probability calculated from phenology alone was 19.9 % (2011), 19.6 % (2012) and 28.0 % (2013), while calculated from spatial overlap alone it amounted to 11.4 % (2011), 12.3 % (2012) and 19.0 % (2013).

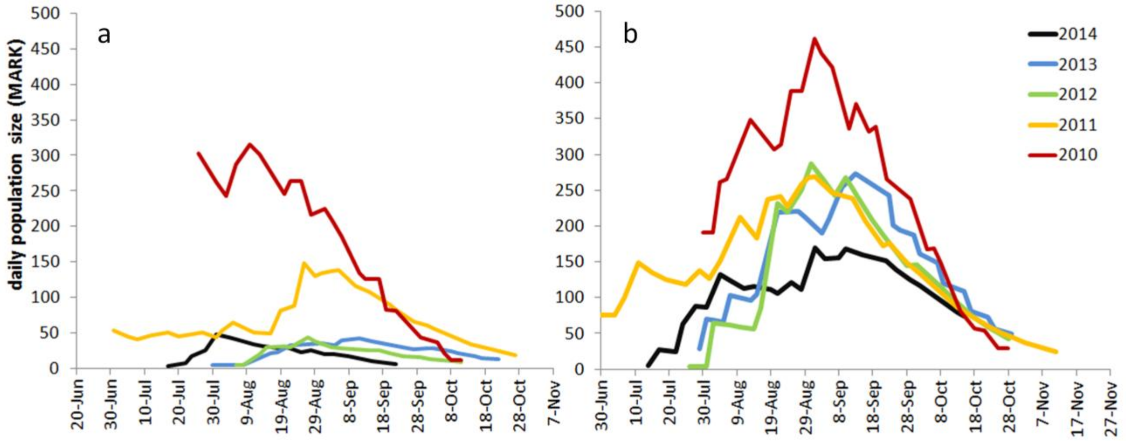

Figure 3 Estimated daily population size of *Ch. montanus* on the Reinsfeld1 (a) and Reinsfeld2 (b) using the program MARK.

Spatiotemporal encounter probability

The mean probability per *Ch. montanus* individual to encounter *Ch. parallelus* (across all grid cells) differed between the periods and years (Table 2). The highest heterospecific encounter probability was found during the first four weeks of the adult season of *Ch. montanus* with a maximum during the second period in 2013 (17.9 %). Afterwards the probability decreased with ongoing season. In 2011, 24 % of the overlapping area had a mean encounter probability greater 10% (across all periods), whereas in 2012 and 2013 this area increased to 27 % and 35 %, respectively. Overall, the spatiotemporal overlap increased from 4.2 % (2011), 4.4 % (2012) to 7.6 % (2013). Hybrids were mainly localized at the edge of the previous year's main distribution of *Ch. montanus* (Figs. 4, 5). The previous year's mean heterospecific encounter probability was significantly higher within a five meter radius of the hybrids' position than in the areas surrounding purebred *Ch. montanus* (ANOVA, $\lambda = 0.2$, $F_{1, 231} = 9.1$, $p = 0.003$; Fig. 6).

Table 2 Mean probability per *Ch. montanus* individual to encounter *Ch. parallelus* (in %) on R2 during seven periods á two weeks from 11 July to 22 October. The (-) marks periods without *Ch. montanus* individuals.

Year	**period 1** 30.6.- 25.7.	**period 2** 26.7.- 09.8.	**period 3** 10.8- 24.8.	**period 4** 25.8.- 09.9.	**period 5** 10.9- 24.9.	**period 6** 25.9.- 08.10.	**period 7** 08.10.- 22.10.	**Mean encounter probability**
2011	8.2	4.0	4.5	4.3	3.0	2.5	2.6	4.2
2012	-	3.0	11.6	4.0	3.2	3.1	1.5	4.4
2013	-	17.9	3.9	7.0	4.8	7.0	4.8	7.6

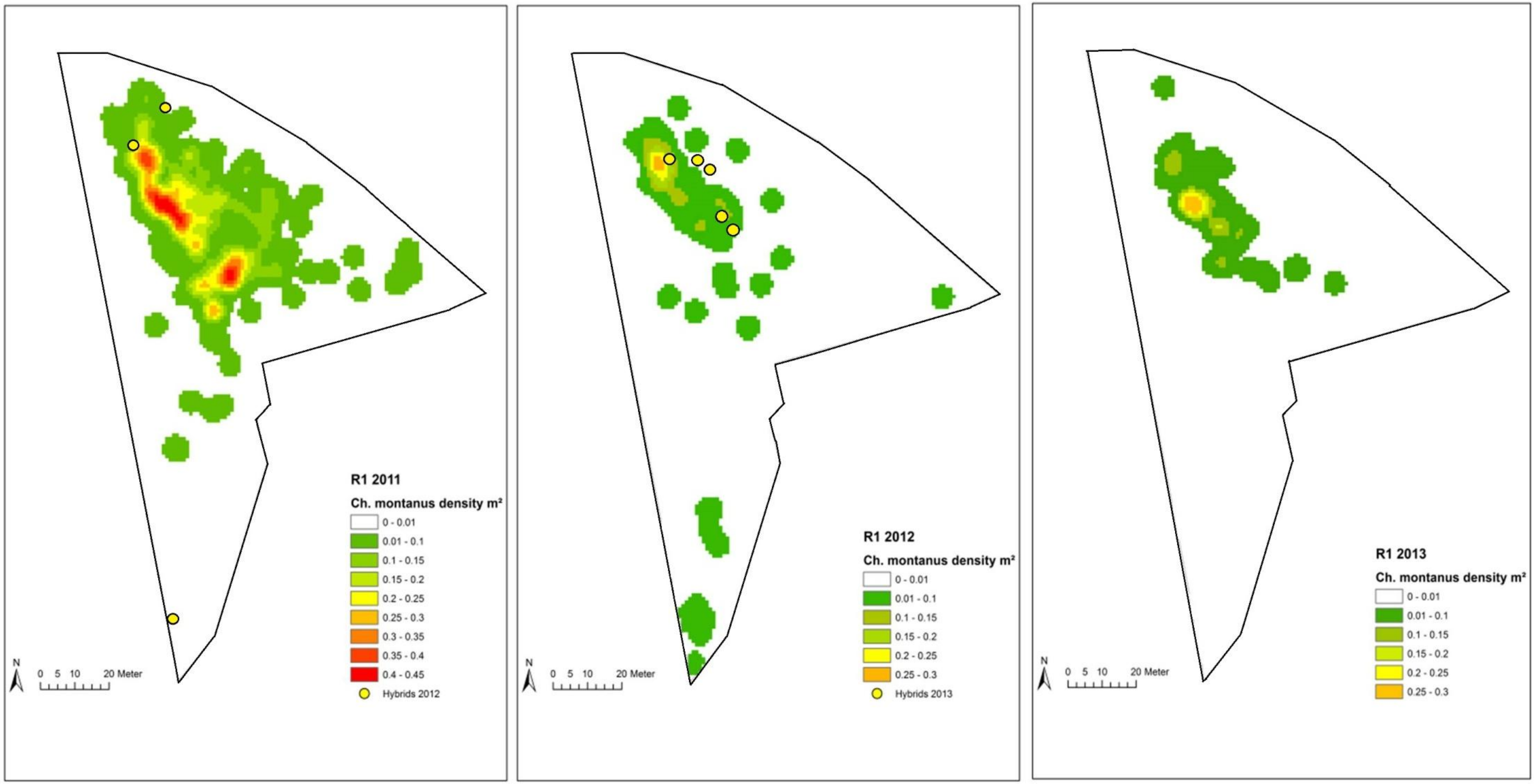

Figure 4 Density distribution of *Ch. montanus* in 2011 to 2013 on Reinsfeld1 including the averaged capture point of hybrids from the following year. There is no data on the capture position of hybrids in 2013 as the individuals of the mark-recapture study have not been genotyped in 2014.

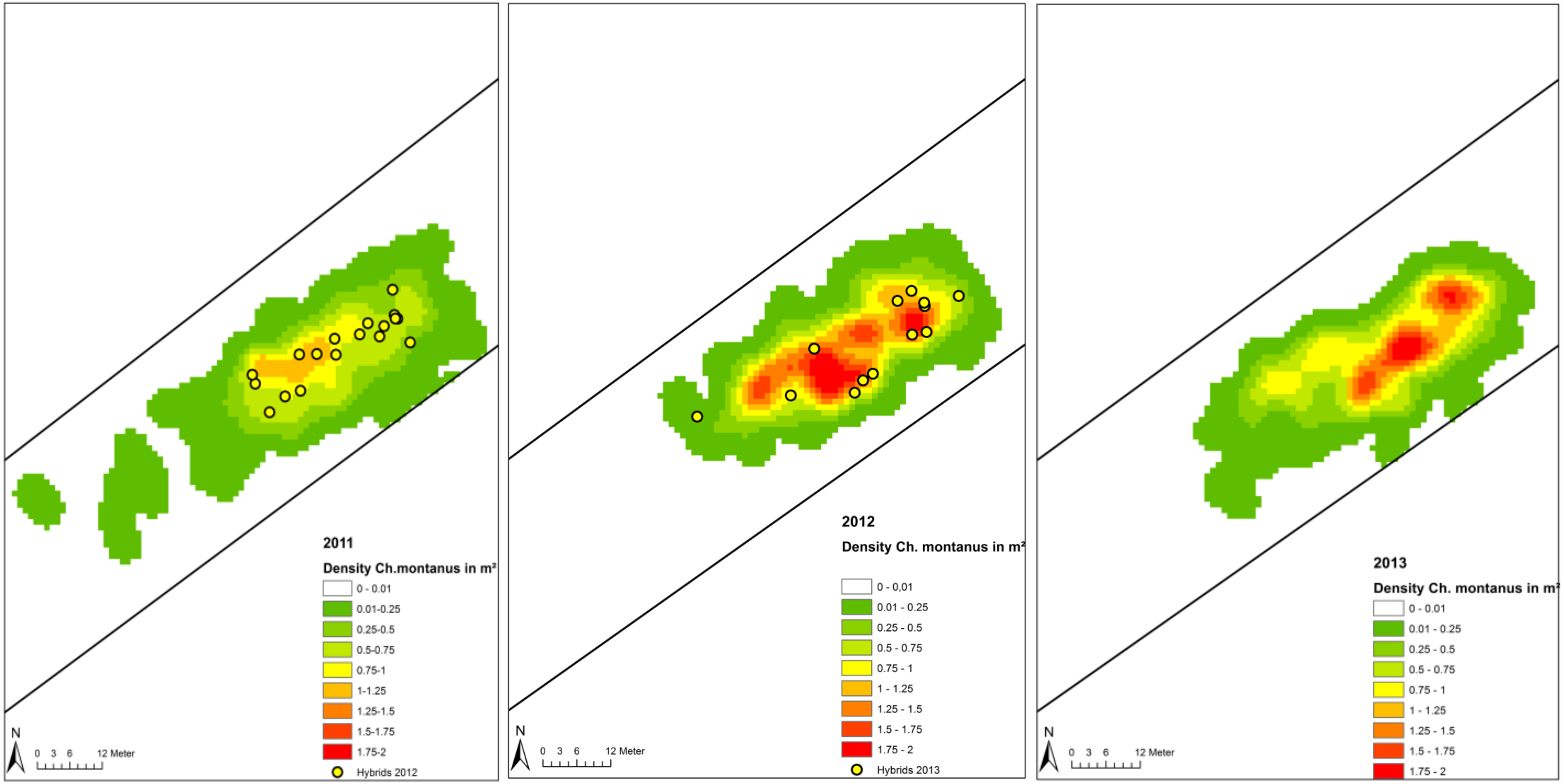

Figure 5 Density distribution of *Ch. montanus* in 2011 to 2013 on Reinsfeld2 including the averaged capture position of hybrids in the following year. There is no data on the capture position of hybrids in 2013 as the individuals of the mark-recapture study have not been genotyped in 2014.

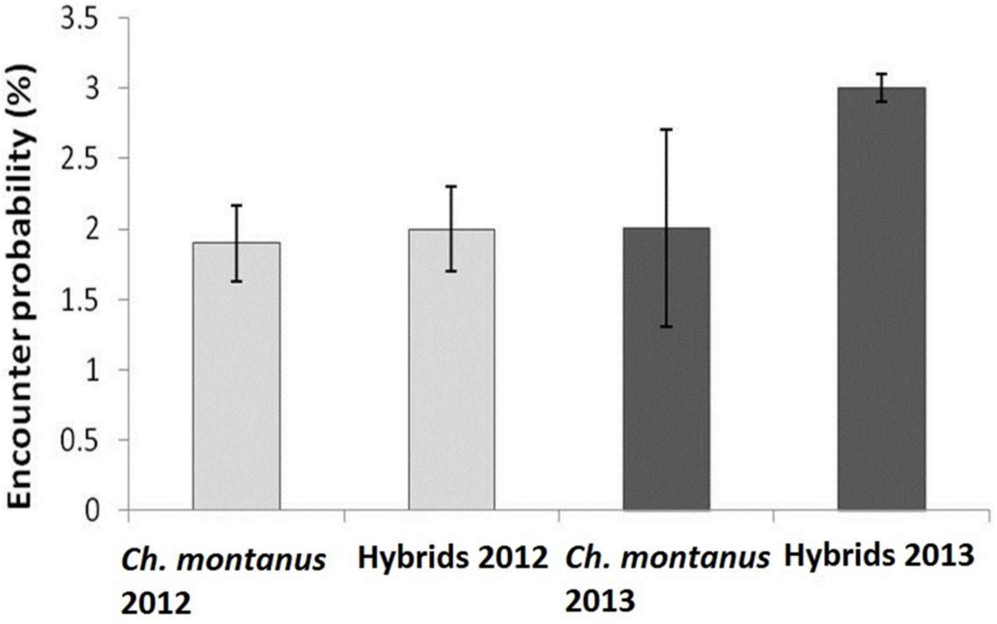

Figure 6 Averaged encounter probability of the previous year (2011, 2012) at the capture position within a radius of five meter for hybrids and the remaining *Ch. montanus* population of the next generation (2012, 2013).

Mean soil moisture at the capture location

The mean soil moisture at the capture locations of *Ch. montanus* (R1: 40-43 %, Fig. S6a, b; R2: 39-40 %; Fig. S7a, b) was significantly higher than at the capture locations of *Ch. parallelus* (R2: 28-29 %; ANOVA, $\lambda = 0.44$, $F_{1,\,61239} = 238.2$, $p < 0.001$). The mean soil moisture at the capture positions of the hybrids varied between 29 % and 38 % (R1: 2012: 29 %; 2013: 36 %; R2: 2012: 38 %; 2013: 33 %; Figs.S6-S7) and was significantly higher than for *Ch. parallelus* (ANOVA, $\lambda = 0.38$, $F_{1,\,6724} = 24.4$, $p < 0.001$), but not significantly different from *Ch. montanus* (ANOVA, $\lambda = 0.56$, $F_{1,\,471} = 1.99$, $p = 0.16$).

Discussion

Our results show that the population decline of *Ch. montanus* correlates strongly with climatic parameters. Droughts during the previous adult season and during the egg stage as well as cloudy conditions during the nymphal period were strongly correlated with population decline. The decreasing population size of *Ch. montanus* also altered the spatiotemporal overlap of both species and increased the heterospecific encounter probability. As mate choice is strongly influenced by encounter probability in this species pair (Chapter II) this increase is likely to be associated with higher hybridization risk. We assume that ongoing climate change and more frequent extreme weather events may further decrease population sizes of

Ch. montanus and may increase the risk of hybridization. In the smaller population (R1) hybridization rate was substantially higher than in the larger population. It has been shown for other taxa that hybridization might ultimately lead to genetic displacement (Schulte *et al.*, 2012). It remains uncertain, whether *Ch. montanus* might have a similar fate in the future, becoming displaced by the habitat generalist *Ch. parallelus*.

Direct effects of climate change and extreme weather events

Climate-mediated range shifts to higher elevations and higher latitudes have been reported for many species (Hickling *et al.*, 2006; Hill *et al.*, 2011; Parmesan, 2006; Warren *et al.*, 2001). For *Ch. montanus* altitudinal shifts are virtually impossible, as it already occurs at the highest elevations of the Hunsrueck Mountains, but it has lost nearly all populations < 400 m asl. Furthermore, it has a very low dispersal ability and its habitats are strongly fragmented (Weyer *et al.*, 2012). The main reason for the strong habitat specialization of *Ch. montanus* is the low drought tolerance of the eggs, which depend upon a permanently high soil moisture (Ingrisch, 1983b). Extreme climatic events such as the three long arid periods observed in April and November 2011, as well as in March 2012 (Barbosa *et al.*, 2012; Bissolli *et al.*, 2012) had direct negative impacts on the *Ch. montanus* populations. However, even other extreme climatic events like extreme precipitation rates during the nymphal phase may lead to population declines. Such extreme weather events are likely to increase in Europe during the next decades (Lehner *et al.*, 2006; Prudhomme *et al.*, 2014). Thus, the time available to compensate population declines between weather extremes is likely to decrease and might drive the populations to extinction. This decline is further promoted by an ongoing habitat degradation due to the lack of habitat management. Both sites have not been mown since we started our project and the accumulation of tangled matted grass is known to negatively affect *Ch. montanus* as well (Weyer *et al.*, 2012). However, the extreme population declines in 2011 and 2012 are more likely to be an effect of the extreme climatic conditions as we would expect a gradual decline by the slow process of habitat degradation. The overall population decline will increase the effects of other threats, such as hybridization, a phenomenon known as the extinction vortex (Gilpin, Soulé, 1986).

Indirect climatic effects on hybridization

Indirect effects of climate change through hybridization are little understood (Chunco, 2014; Sánchez-Guillén *et al.*, 2014). An asynchronic response to climate change *may affect the relative frequencies* of species and alter their coexistence (Heard *et al.*, 2012; Sánchez-

Guillén *et al.*, 2014). The two grasshopper species studied differed strongly in their population response to climatic extremes. While *Ch. montanus* strongly declined after arid periods, *Ch. parallelus* populations remained almost stable. Habitat specialists like *Ch. montanus* are more sensitive to changing environmental conditions than habitat generalists as they have lower tolerances (Gilchrist, 2000), which will change the hybridization dynamics among both species. The remaining *Ch. montanus* population becomes increasingly restricted to the wettest areas, whereas the *Ch. parallelus* population can expand and immigrate into the *Ch. montanus* population. The changes in the relative frequencies of both species as well as the changing spatial overlap increases the probability for *Ch. montanus* to encounter *Ch. parallelus* and thus for hybridization (Chapter II). In our study, hybrids were mainly found at the edge of the *Ch. montanus* populations, in areas with high heterospecific encounter probabilities of the previous year. This result is surprising given the ability of the grasshoppers to move throughout their nymphal and adult stage. However, it is known that adults of this species do not move large distances (Weyer *et al.*, 2012) and this is also likely for the nymphs, which spend more time in feeding than in locomotion.

The role of premating barriers

The two studied species have large overlapping ranges, suggesting that hybridization interactions may occur in many populations, similar to a mosaic hybrid zone (Köhler, 2013). However, hybridization seems to be minimized by the low spatiotemporal overlap of both species, caused by differing microhabitat preferences and phenologies. The combination of spatial and temporal niche divergence reduces hybridization risk dramatically. Spatial population overlap alone would suggest an 11-19 % heterospecific encounter probability and temporal overlap a 20-28 % probability. However, both dimensions together reduce the mean heterospecific encounter probabilities on R2 to 4.2 % (2011), 4.4 % (2012) and 7.6 % (2013), which is much closer to our observed hybridization rates of 7.4 % (2012) and 6.0 % (2013). In the smaller population (R1) hybrids already have reached frequencies between 15.6 % and 19.6 %. This corroborates the hypothesis that the population decline of *Ch. montanus*, which was initially triggered by climatic factors and habitat deterioration, now increasingly causes an additional threat from hybridization. Even under favorable conditions for *Ch. montanus*, the populations of both species are likely to fluctuate permanently and thus hybridization equilibria are probably also under permanent fluctuation.

Due to the earlier adult season of *Ch. parallelus*, heterospecific encounter probabilities were highest during the first month of the adult season of *Ch. montanus*. Even though freshly molted individuals might not be sexually receptive immediately (Kriegbaum, 1988), hybridization risk probably peaks as soon as they are receptive. Interestingly, *Ch. parallelus* varied strongly in phenology in the overlapping area of both populations. In 2011 the highest daily population size of *Ch. parallelus* was two months earlier than for *Ch. montanus*. In 2012, the difference was only one month and in 2013 only two weeks. This phenology shift was much less pronounced when considering the complete spatial distribution of the *Ch. parallelus* population, suggesting that this species might react much more sensitive to climatic fluctuations under ecological unfavorable conditions. A possible explanation might be a delayed egg development under wetter and colder conditions. This shows that although phenology may play an important role as reproductive barrier, it can fluctuate dramatically in response to environmental changes (Parmesan, 2006; Parmesan, Yohe, 2003; Rosenzweig *et al.*, 2007).

The differing water requirements of the eggs of both species (Ingrisch, 1983b) are thought to be a major reason for the spatial segregation of both species (Köhler, 2013). Our study corroborates this hypothesis. *Ch. parallelus* preferred areas with lower soil moisture than *Ch. montanus*, but it had a broader ecological amplitude and also entered areas of higher soil moisture. This suggests that the ecological barrier alone would not be sufficient to prevent admixture. However, a decreasing soil moisture after long arid periods would probably increase *Ch. parallelus* densities in the center of the habitat of *Ch. montanus*. Interestingly, hybrids were mainly found in areas of intermediate soil moisture. It remains unknown, whether this is a consequence of intermediate water requirements, or if it is a secondary result caused by the higher hybridization probability in zones of highest spatial overlap, which are likely to have an intermediate soil moisture.

Conclusion

Our results show that extreme climatic events present a major threat for habitat specialists such as *Ch. montanus* both directly by reducing reproductive success and indirectly by increasing heterospecific encounter probabilities, which are assumably associated with a higher hybridization risk. The increasing probability of extreme climatic events as predicted by climate models represents a major threat to small and fragmented populations of many wetland specialists. It remains to be studied, whether hybridization risk further increases with decreasing population sizes. Other studies have shown before that anthropogenic disturbances can affect existing reproductive barriers between syntopic populations and promote hybridization (Seehausen *et al.*, 2008a). Reproductive barriers remain effective as long as syntopic populations fluctuate around an equilibrium, but we hypothesize that as soon as population trends lead to a directional change of the relative frequencies, reproductive barriers may break down and the smaller population will be “genetically swamped”.

Acknowledgements

This study was funded by the German Research Foundation (DFG, GRK 1319) in the interdisciplinary graduate school ‘Cooperation of Science and Jurisprudence in Improving Development and Use of Standards for Environmental Protection – Strategies for Risk Assessment and Management’. The “Struktur- and Genehmigungsdirektion Nord” permitted the collection of samples and access to nature reserves. We also acknowledge Marielle Meyer and Marco Kranz for data collection in the field as well as Sabine Naber and Karin Fischer for laboratory assistance.

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Supplementary data

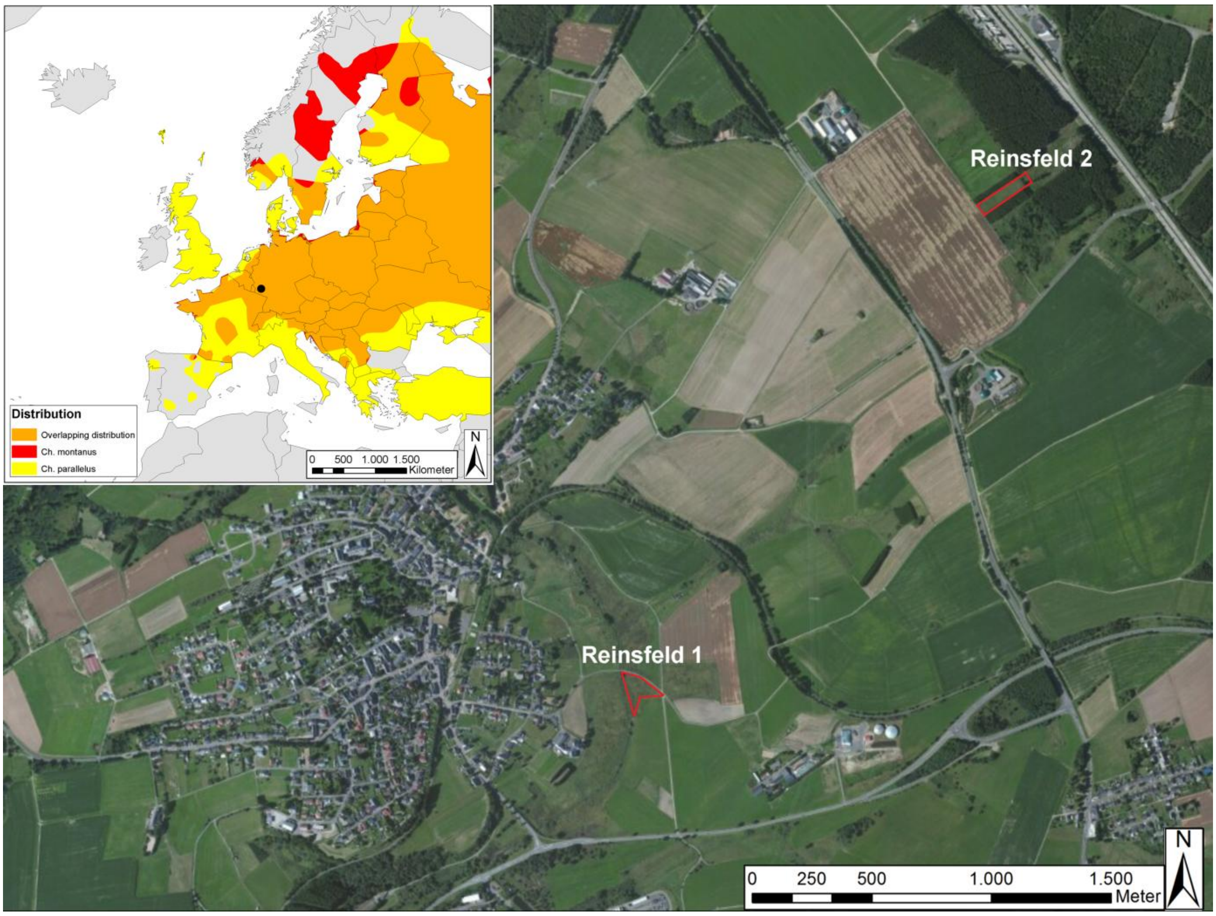

Figure S1 Top left: Distribution of *Ch. montanus* (red), *Ch. parallelus* (yellow) and the overlapping distribution of both species(orange) (modified after Kleukers 1997). The region of the two study sites Reinsfeld1 and Reinsfeld 2 in the Hunsrück mountains (Rhineland-Palatinate, Germany) are marked with a black dot. Underlying map: Location (red) of both study sites.

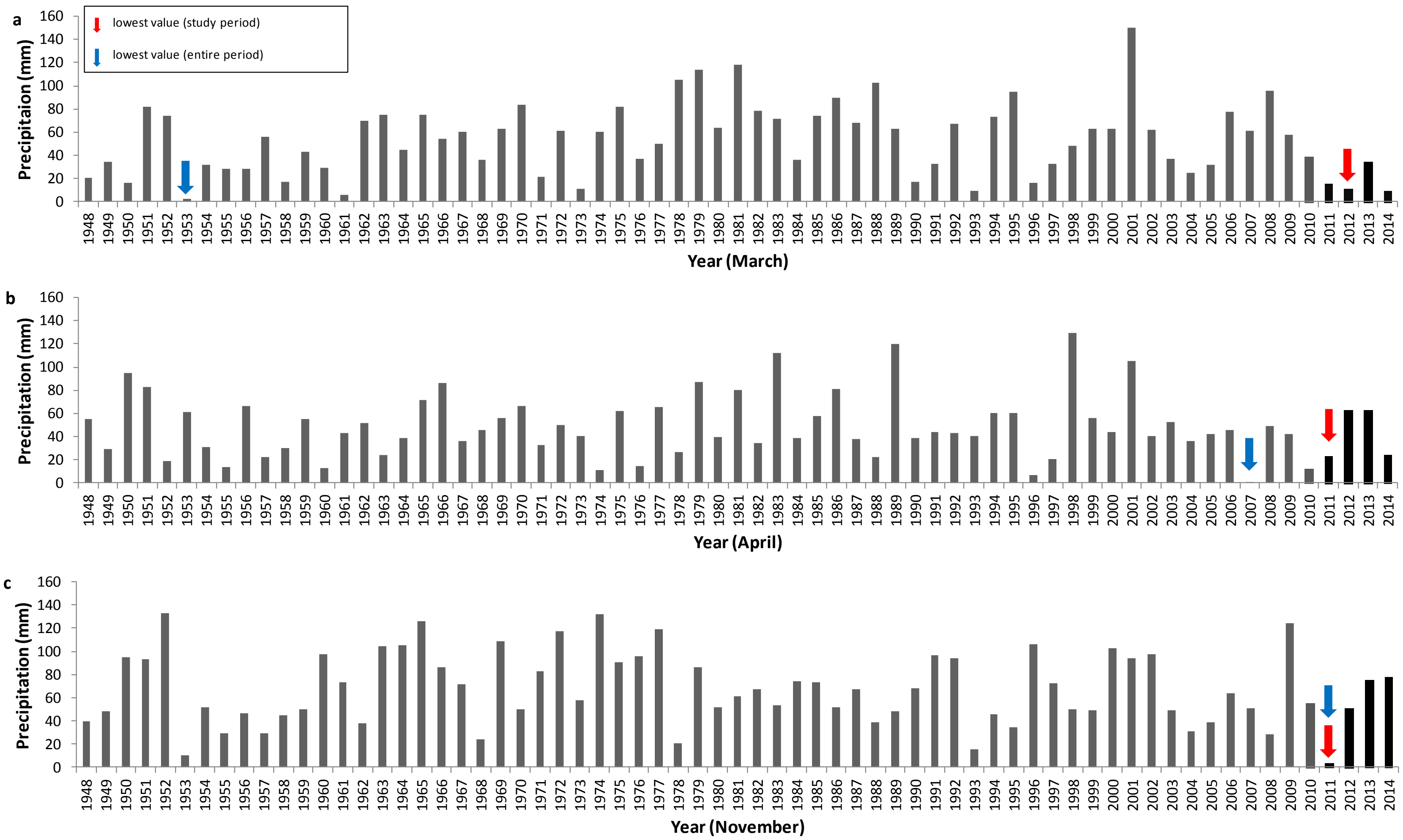

Figure S2 Overview of the precipitation rate in selected month (a. March, b. April, c. November; egg phase Ch. montanus) from 1948 to 2014. The lowest precipitation rates during the entire period are marked with blue arrows, the lowest precipitation rates during the study period (2011-2014; black bars) are marked with red arrows.

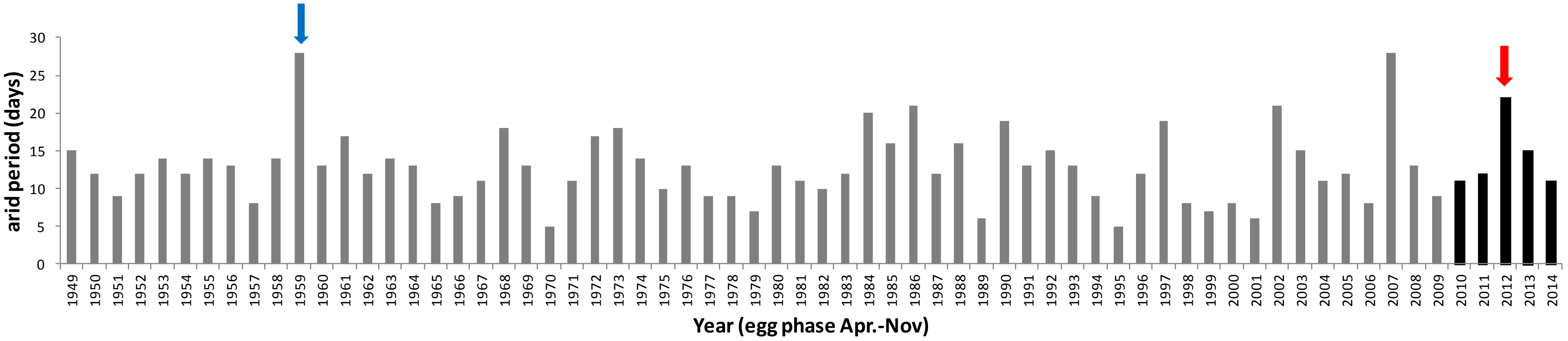

Figure S3 Overview of the longest arid period during the egg phase of Ch. montanus) from 1948 to 2014. The longest arid period during the entire period is marked with a blue arrow, the longest rid period during the study period (2011-2014; black bars) is marked with a red arrow.

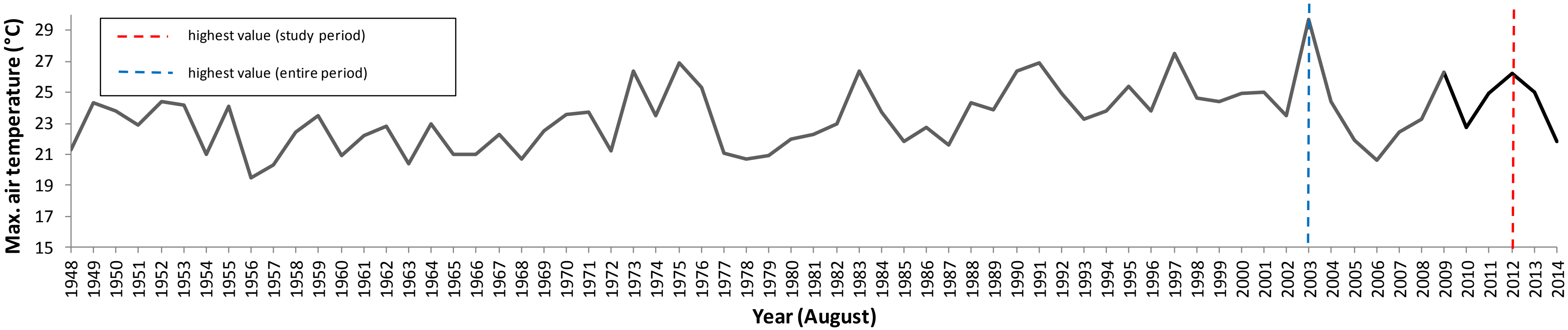

Figure S4 Temperature profile of the month August from 1948 to 2014. The highest value during the entire period is marked with a blue line, the highest value during the study period (2011-2014; black bars) is marked with a red line.

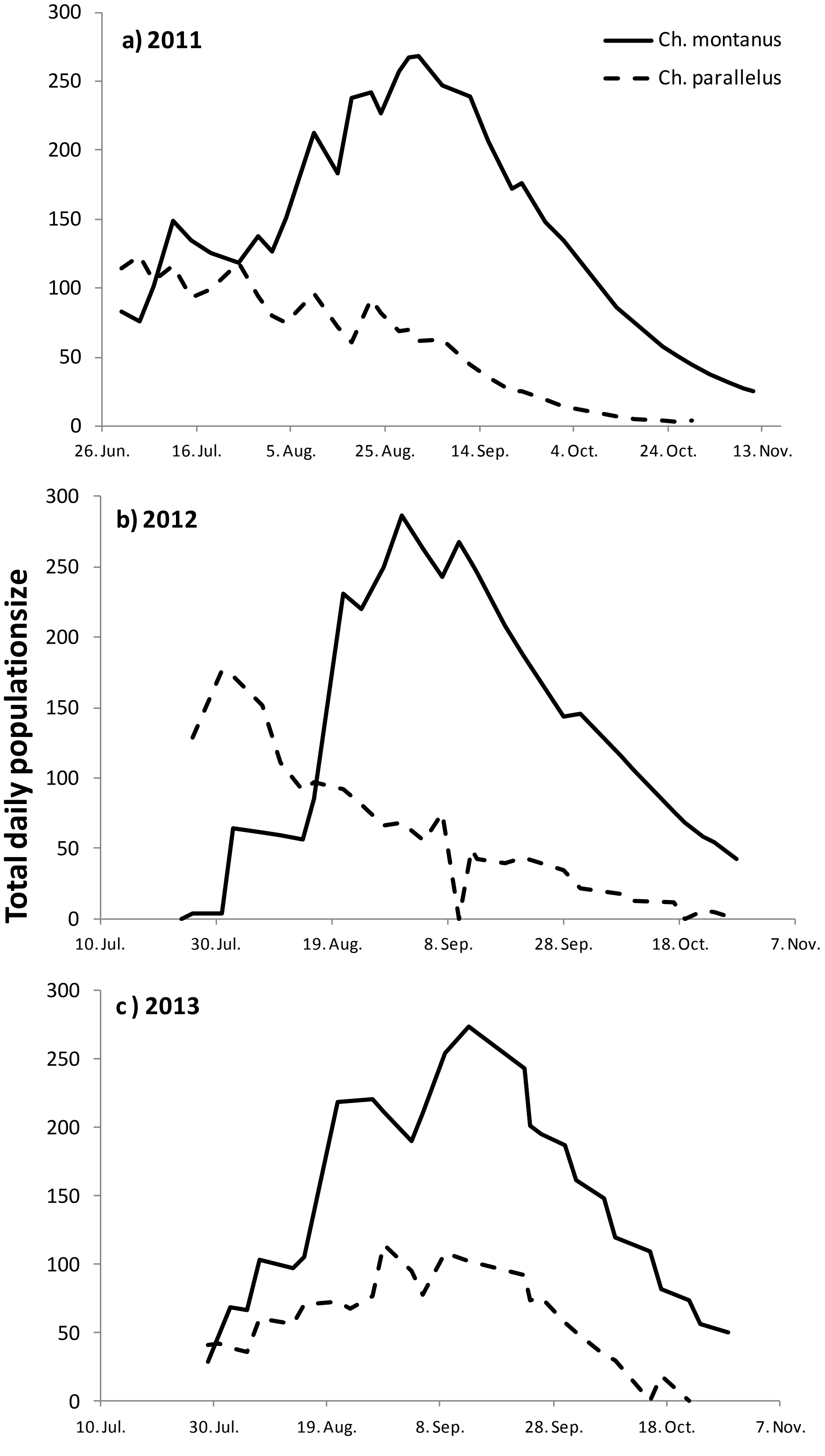

Figure S5 Total daily population size of *Ch. montanus* and *Ch. parallelus* within the overlapping habitat of Reinsfeld2 (a=2011; b=2012; c=2013).

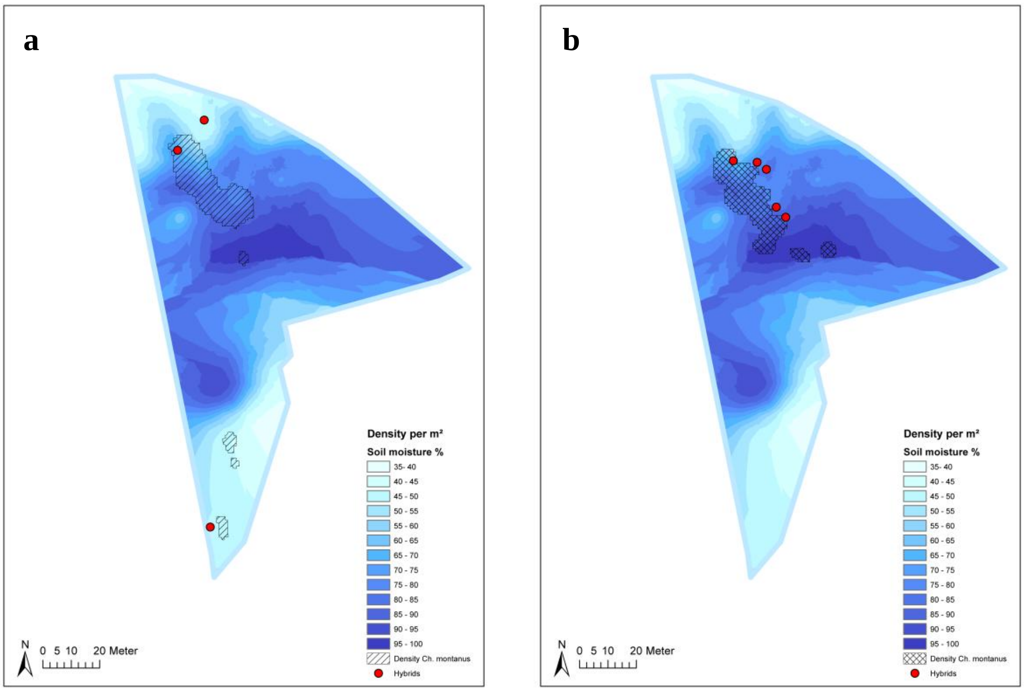

Figure S6 Soil moisture distribution on R1 with the maximal distribution boundary of *Ch. montanus* for the year 2012 (a) and 2013 (b) and the corresponding hybrids.

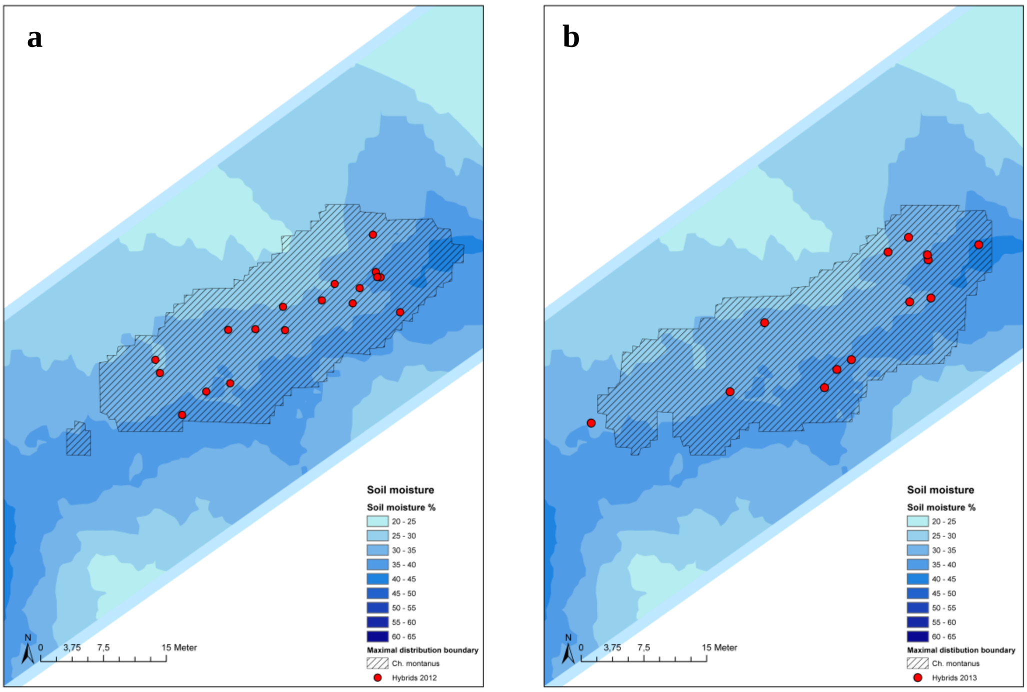

Figure S7 Soil moisture distribution on R2 with the maximal distribution boundary of *Ch. montanus* for the year 2012 (a) and 2013 (b) and the corresponding hybrids.

Table S1 Performance test of three genetic programs (adegenet, NewHybrids and Structure) on hybrid detection in a simulated data set. In total 200 hybrids (50 per hybrid class, F1, F2, Backcoss with *Ch. montanus* = Bmon; Backcoss with *Ch. parallelus* = Bpara) were previous simulated in HYBRIDLAB 1.1 (Nielsen et al. 2006) using purebred individuals from a previous analysis of the two study sites Reinsfeld 1 and Reinsfeld 2 (43 *Ch. montanus*, 40 *Ch. parallelus*). A threshold value of q = 0.91 was used for hybrid detection in STRUCTURE.

		adegenet		**NewHybrids**		**STRUCTURE**	
Category	Sample size	mis-assigned	%	mis-assigned	%	mis-assigned	%
Hybrid	**200**	**12**	**6.0**	**5**	**2.5**	**19**	**9.5**
F1	50	0	0.0	0	0.0	0	0.0
F2	50	0	0.0	0	0.0	2	4.0
Bmon	50	4	8.0	4	8.0	7	14.0
Bpara	50	8	16.0	1	2.0	10	20.0
Purebred	**83**	**21**	**25.3**	**29**	**34.9**	**26**	**31.3**
Total	**283**	**31**	**11.0**	**34**	**12.0**	**45**	**15.9**
Correct assignment			**89.0**		**88.0**		**84.1**

Chapter IV

The role of hybridization in biodiversity conservation – Legal approaches and challenges in the International, EU and German national law

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Unpublished manuscript

Table of Contents

List of abbreviations

AC	Animals Committee (CITES)
Art.	Article
BArtSchV	Bundesartenschutzverordnung; German Federal Species Protection Regulation
Bern Convention	Convention on the Conservation of European Wildlife and Natural Habitats (1979)
BfN	Bundesamt für Naturschutz (Germany)
BGBl.	Bundesgesetzblatt; German Federal Law Gazette
BJagdG	Bundesjagdgesetz; Federal Game Law
BNatSchG	Bundesnaturschutzgesetz; German Federal Nature Conservation Act
BVerfGE	Entscheidungen des Bundesverfassungsgericht; Decisions of the Federal Constitutional Court
BWildSchV	Bundeswildschutzverordnung; Federal Wildlife Regulation
CBD	Convention on Biological Diversity (1992)
CITES	Convention on International Trade of Endangered Species and of Wild Flora and Fauna (1973)
CJEU	European Court of Justice
COM	Commission (EU)
CoP	Conference of the Parties (CITES)
COP	Conference of the Parties (CBD)
DEC	Decision (CBD)
EC	European Commission
ETS	European Treaty Series
EEC	European Economic Community
EU	European Union
GG	Grundgesetz für die Bundesrepublik Deutschland; German constitution
GVG	Gerichtsverfassungsgesetz, German judicature act
Habitats Directive	Council Directive 92/43/EEC on the Conservation of Natural Habitats of Wild Flora and Fauna (1992)
IO	international organization
IAS	Invasive alien species
IUCN	International Union for Conservation of Nature
LCIE	Large Carnivore Initiative for Europe
LIFE	L' Instrument Financier pour l' Environnement (EU)
MEA	multilateral environmental agreement
NABU	Naturschutzbund Deutschland e. V.
Res. Conf.	Resolution Conference
Rev. CoP	Revised Conference of Parties (CITES)
Rio Declaration	Rio Declaration on Environment and Development (1992)
SBSTTA	Subsidiary Body on Scientific,, Technical and Technological Advice (CBD)
SSC	Species Survival Commission (IUCN)
Stockholm Conference	United Nation Conference on Human Environment
TFEU	Treaty on Functionality of the European Union

T-PVS	Working document of the Bern Convention
UN	United Nations
UNCHE	United Nation Conference on Human Environment (Stockholm Conference, 1972)
UNEP	United Nations Environment Programme
UNTC	United Nations Treaty Collection
UNTS	United Nations Treaty Series
USA	United States of America
VCTL	Vienna Convention on the Law of Treaties
WCED	World Commission on Environment and Development

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Introduction

The ongoing decline of biodiversity is an increasing global problem for conservationists.[1] Anthropogenic disturbances such as habitat loss, the introduction of non-native species, pollution and climate change represent the main threats to biodiversity.[2] Some of these disturbances form another major threat: hybridization.[3] It is a fascinating phenomenon which so far particularly caught the attention of evolutionary biologists. In light of biodiversity loss, it has also become an increasingly important field for conservation biologists during recent years.[4] A legally binding definition of hybridization is missing and even biologists are reluctant to agree on a single definition.[5] The most widely used biological approach defines hybridization as the interbreeding of two genetically distinct individuals which belong to different populations or species.[6] In addition, neither a legal nor a definite biological definition of hybrids is clarified yet.[7] On the basis of the aforementioned definition only the direct offspring (first generation) of two genetically distinct individuals would be counted as hybrid whereas second generation hybrids or backcrosses are neglected.[8]

Currently, the true extent of hybridization in the wild is not precisely known and so is the number of hybridizing taxa. The consequences of hybridization are difficult to predict and depend on the species involved.[9] In face of biodiversity conservation, it is necessary to distinguish between natural and anthropogenic hybridization. Natural hybridization facilitated by altering environmental conditions is recognized as a common evolutionary process important to trigger speciation.[10] It is assumed that 25 % of plants and

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[1] *Secretariat of the Convention on Biological Diversity* (2010) Global Biodiversity Outlook 3, 17 *and* (2014) *Global Biodiversity Outlook 4, 24-25*.

[2] *Ibid.*, 9.

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[4] *Harrison*, Hybrids and Hybrid Zones: Historical Perspective, in *Richard G. Harrison*, Hybrid Zones and The Evolutionary Process (1993) 3-12, 3; *Abbott et al.*, Hybridization and speciation, Journal of Evolutionary Biology 26.2 (2013): 229-246.; *Rhymer/ Simberloff*, Annual Review of Ecology and Systematics (1996): 83-109; *Seehausen*, Hybridization and adaptive radiation, Trends in ecology & evolution 19.4 (2004): 198-207; *Seehausen et al.*, Genomics and the origin of species, Nature Reviews Genetics 15.3 (2014): 176-192; *Chunco*, Ecology and Evolution 4.10 (2014): 2019-2031; *Allendorf/ Leary/ Spruell/ Wenburg*, The problems with hybrids: setting conservation guidelines, Trends in Ecology & Evolution 16.11 (2001): 613-622.

[5] *See* Definition of hybridization in the Factual Background and Conclusion and Summary of this study.

[6] *Seehausen et al.*, Nature Reviews Genetics 15.3 (2014): 176-192, 181.

[7] *Harrison*, in *Richard G. Harrison*, Hybrid Zones and The Evolutionary Process (1993): 3-12, 5-6.

[8] *See* the Definition of hybridization in the Factual Background chapter 1.

[9] *Björklund*, The unpredictable impact of hybridization, Journal of Evolutionary Biology 26 (2013): 274-275, 275.

[10] *Grant/ Grant*, Hybridization of bird species, Science 256.5054 (1992): 193-197, 197.

10% of European animals hybridize naturally.[11] Whether it has positive or negative effects on biodiversity, natural hybridization is an evolutionary effect of a naturally changing environment.[12]

By contrast, anthropogenic hybridization is a consequence of human interventions which can be observed in terrestrial as well as in aquatic habitats.[13] Anthropogenic causes of hybridization could be direct or indirect. Direct causes are the unintentional and the intentional infiltration of species (invasive or domesticated species) by humans. Species that are naturally separated due to insuperable geographical barriers are brought into contact directly by humans. If the introduced species is more adaptable to the new habitat than the native species, one consequence may be the genetic displacement of the native species. By contrast, indirect causes are for example human interventions which induce climate change or habitat change. This in turn may result into a secondary contact of naturally separated species or populations by breaking up ecological or geographical barriers and therefore, it may foster hybridization.[14] Anthropogenic hybridization is assumed to cause species extinction and thus threaten biodiversity. Particularly when rare species are involved, the risk of genetic displacement increases.[15]

It took a long time until humans realized that their actions may harm ecosystems and biodiversity. The interdependences between single components are complex and difficult to predict. It is thus not surprising that for a long time hybridization has not played a major role in conservation. The relevance of hybridization for biodiversity and its conservation has emerged slowly and gains only little to no attention by decision makers, media and civil society.

Aim of the study

The aim of this study is to outline the legal status of hybridization and hybrids. Furthermore, existing legal uncertainties dealing with the terms ‘hybridization’ and ‘hybrid’ in different levels of law are analyzed. The study provides an overview of the historical background of hybrids becoming a part of legal instruments. Additionally, it

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[11] *Mallet,* Hybridization as an invasion of the genome, Trends in Ecology & Evolution 20.5 (2005): 229-237, 229.

[12] *See also* the Consequences of hybridization in chapter 1.

[13] *Mallet,* Trends in Ecology & Evolution 20.5 (2005): 229-237, 231; *Epifanio/ Nielsen*, The role of hybridization in the distribution, conservation and management of aquatic species, Reviews in Fish Biology and Fisheries 10.3 (2000): 245-251, 245.

[14] *Seehausen/ Takimoto/ Roy/ Jokela,* Speciation reversal and biodiversity dynamics with hybridization in changing environments, Molecular Ecology (2008): 17(1), 30-44, 30.

[15] *Allendorf/ Leary/ Spruell/ Wenburg,* The problems with hybrids: setting conservation guidelines, Trends in Ecology & Evolution 16.11 (2001): 613-622, 613; *Rhymer/ Simberloff,* Annual Review of Ecology and Systematics (1996): 83-109, 83.

assesses the role of hybrids in order to potentially amend existing or adopting new legal instruments and to investigate the potential differences dealing with hybrids between international, European and national law. Finally, the study aims to emphasize the challenges which occur with setting guidelines dealing with hybrids and how they could be handled.

Scope of the study

The present study focuses on anthropogenic hybridization, as it is this category which has a particular negative impact on biodiversity.[16] In contrast, conservationists are concerned by natural hybridization to a far lesser degree, since it is an evolutionary process which is important to ensure the persistence of biodiversity in the future.[17] Moreover, only animal hybridization processes in the wild (excluding artificial hybridization) are assessed here.

The present study is divided into five chapters. The first chapter gives a short overview of the factual background of hybridization and hybrids and thus provides the basis for the legal assessment. The understanding of the ecological coherences of hybridization is essential for an effective evaluation of the existing legal instruments and the development of recommendations for future amendments.

In the second chapter, the role of hybrids in international environmental law is analyzed. First, the regulatory framework of international law applicable to hybridization and hybrids is examined. The two most important international conventions in the context of hybridization are then assessed with regard to the legal obligations contained therein. Also non-binding approaches are evaluated, taking into account that they might present the basis for future legal developments.

The focus of the third chapter is on European environmental law. Due to the supranational structure of the European Union (EU), European law is characterized by particular legal effects (e.g. concerning its supremacy vis-à-vis domestic law as well as enforcement) which make it necessary to distinguish it from "traditional" international law. Thereafter, implementation of the sole international convention that contains a resolution of hybrids within the EU legal system is analyzed. In the following subsection, other relevant policies

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[16] *Rhymer/ Simberloff*, Annual Review of Ecology and Systematics (1996): 83-109, 103.

[17] *Genovart*, Natural hybridization and conservation, Biodiversity and Conservation 18.6 (2009): 1435-1439, 1437.

applicable to biodiversity are examined with regard to their relevance for hybridization and hybrids.

The fourth chapter deals with the role of hybridization and hybrids at the national level. Germany is chosen as a Member State to the EU as well as a party to the examined international conventions.

A. Factual Background

Definition of hybridization and hybrid

Based on different species concepts, defining hybridization and hybrids constitutes a serious challenge for biologists.[18] It is beyond the scope of this study to discuss all existing species concepts. The Biological Species Concept defines species as the follows:

> Species are groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1995).[19]

Other authors argue in favor of less strict approaches.[20] They emphasize that species are characterized by "substantial but not necessarily complete reproductive isolation*"*.[21] This definition is particularly suitable to serve as yardstick for the further analysis in light of the fact that it has also been referred to by the States parties to the Convention on the International Trade of Endangered Species of Wild Flora and Fauna (CITES).[22] Complete reproductive isolation entirely prevents hybridization and clearly separates one species from another. The situation is different if reproductive isolation is incomplete and hybridization occurs. Under the aforementioned species concept the emergence of viable

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[18] *Harrison,* in *Richard G. Harrison,* Hybrid Zones and The Evolutionary Process (1993): 3-12, 4-6; Agapow *et al.*, The impact of species concept on biodiversity studies, The Quarterly Review of Biology, 79.2 (2004): 161-179, 162.

[19] *Coyne/ Orr,* Speciation: Species: Reality and Concepts, Sinauer Associates, Inc. (2004) 9-48, 27.

[20] *Coyne/ Orr,* Sinauer Associates, Inc. (2004) 9-48, 30; Agapow *et al.*, The Quarterly Review of Biology, 79.2 (2004): 161-179, 172.

[21] *Coyne/ Orr,* Sinauer Associates, Inc. (2004) 9-48, 30; Reproductive isolation is assured through isolating or reproductive barriers which are defined by the authors as: "*those biological features of organism that impede the exchange of genes with members of other population*."

[22] *CITES* is an international agreement between governments which regulates the trade of specimen with the aim to ensure their survival in the wild. It entered into force at 1st July 1975. In Annex 5 of the Resolution Conference 9.24 (Rev. CoP16) on the Criteria for the amendment of Appendices I and II (Bangkok, Thailand, 03-14 March 2013) the States parties to the Convention determined that in this case: "*'Species' and 'subspecies' refer to the biological concept of a species, and do not require any further definition*." Resolutions represent non-legally binding documents which provide guidelines for the implementation of this Convention.

and fertile offspring via hybridization of two different species is virtually impossible.[23] Thus, it is debatable whether hybridizing populations, based on incomplete reproductive isolation, can be counted as different species if they produce fertile hybrid offspring.[24] In consequence of this problem, different definitions of hybridization have been suggested. One of the most recently published definitions states that hybridization is the:

> Mating between individuals that belong to distinct species or populations. If post-mating isolation is incomplete, hybridization leads to the introgression of genes from one population to another.[25]

Another definition excludes the term `species´ from the definition, saying that hybridization is the:

> interbreeding of individuals from genetically distinct populations, regardless of the taxonomic status of the populations.[26]

Both definitions insist on genetic differences between both individuals involved in hybridization. Furthermore, they indicate that the species concept should not be regarded as a rigid category, but rather as a continuum that is influenced and changed by altering living conditions. It should furthermore be taken into account that hybridization does not necessarily lead to hybrid offspring.[27]

Furthermore, no generally agreed definition of hybrids exists.[28] Offspring which emerges directly as a result of hybridization are hybrids of the first generation (F1-hybrids). However, also F1-hybrid crosses result in a new hybrid generation (F2-hybrids) even if the reproduction of two hybrids is not applicable to the aforementioned definition of hybridization. Against this background, it is submitted that the following definition is the most suitable one and may best serve as reference for the following study: *a hybrid is defined as the offspring of two interbreeding individuals from genetically distinct populations including even further hybrid generations (F2; Backcrosses with parental species).*

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[23] *Ibid*., 30; Agapow *et al.*, The quarterly review of biology, 79.2 (2004): 161-179, 171.

[24] Agapow *et al.*, The quarterly review of biology, 79.2 (2004): 161-179.

[25] *Seehausen et al.*, Nature Reviews Genetics 15.3 (2014): 176-192, 181.

[26] *Rhymer/ Simberloff*, Annual Review of Ecology and Systematics (1996): 83-109, 84; *Allendorf/ Leary/ Spruell/ Wenburg*, Trends in Ecology & Evolution 16.11 (2001): 613-622, 621.

[27] *Hochkirch*, Hybridization and the origin of species, Journal of Evolutionary Biology 26.2 (2013): 247-251, 247.

[28] *Harrison*, in *Richard G. Harrison*, Hybrid Zones and The Evolutionary Process (1993): 3-12, 5-6.

Anthropogenic Causes for hybridization

Changing environmental conditions due to habitat loss and the introduction of (invasive, domesticated) species outside their natural range as well as climate change lead to new species compositions. Consequently, existing reproductive barriers can break down and therefore, may cause hybridization in originally separated and distinguishable populations or species. However, it must be taken into account that hybridization may mainly occur during a transition period and might stop as soon as new reproductive barriers have evolved, one species has been displaced or a new species has emerged from the hybrid form. Anthropogenic hybridization thus results from environmental disturbances by humans, and only occurs if reproductive barriers between the individuals or populations involved are incomplete.

Consequences of hybridization

Depending upon the genetic relationship of the populations concerned, the consequences of hybridization differ to a significant degree. On the one hand, hybridization may facilitate the adaptation of species to environmental changes when new alleles are implemented in the gene pool.[29] Such new adaptations in turn may ensure their survival in altering habitats.[30] Furthermore, an admixture of genomes leads to an increasing genetic diversity which could prevent small and isolated populations from inbreeding depression and, ultimately, from extinction.[31] On the other hand, introgression could lead to genetic displacement and genetic extinction of populations or even entire species particularly if one parental species is rare.[32] Hybridization processes without hybrid offspring also have an impact on population fitness and could weaken a population due to direct and indirect fitness costs.[33] If hybrids have a higher fitness than parental species due to a better adaptation to the prevalent environmental conditions, hybridization may cause hybrid

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[29] *Rieseberg et al.*, Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301.5637 (2003): 1211-1216, 1216.

[30] *Baskett/ Gomulkiewicz*, Introgressive hybridization as a mechanism for species rescue, Theoretical Ecology 4.2 (2011): 223-239.

[31] *Mallet*, Hybrid speciation, Nature, 446.7133 (2007): 279-283, 279; *Arnold/ Sapir/ Martin*, Genetic exchange and the origin of adaptations: prokaryotes to primates, Phil. Tran. R. Soc. B 363 (2008), 2813-2820, 2813; *Abbott et al*, Hybridization and speciation, Journal of Evolutionary Biology (2013), 229-246, 241; *Baskett/ Gomulkiewic*, Theoretical Ecology 4.2 (2011), 223.

[32] *Wolf/ Takebayashi/ Rieseberg*, Predicting the risk of extinction through hybridization, Conservation Biology 15.4 (2001): 1039-1053, 1052.

[33] *Rhymer/ Simberloff*, Annual Review of Ecology and Systematics (1996): 83-109, 84; *Hochkirch/ Gröning/ Bücker*, Sympatry with the devil: reproductive interference could hamper species coexistence, Journal of Animal Ecology 76.4 (2007): 633-642, 639.

speciation, resulting in the formation of a new species.[34] Therefore, hybridization may result in new species compositions that can have a negative or positive impact on biodiversity and the species involved.

Detection of hybrids and hybridization processes

Historically, hybridization processes in the wild were usually first recognized when intermediate morphotypes were identified as hybrid offspring or a population decline was observed. Nowadays, several molecular approaches for the detection of hybrids exist that have partly replaced the less reliable method of morphological identification of hybrids.[35] Phenotypic changes could give evidence for hybridization processes which have to be genetically confirmed to prove ongoing hybridization processes.[36]

B. International environmental law in the context of hybridization

I. General Information on international environmental policy and law

Ecosystems including their biodiversity are of enormous global importance and essential for human well-being. The main threats to biodiversity such as invasive species, climate change and habitat fragmentation are global problems with "a transnational nature".[37] However, it is precisely these threats that also trigger hybridization.[38] International trade and traffic cause the introduction of species to new environments, thereby ultimately abetting hybridization. In addition, also climate change leads to range shifts of species and may lead to new species compositions which in turn may result in hybridization. Due to the transboundary effects of these processes, it is necessary to examine the role of hybridization in international environmental law. In the present context, international treaties and soft law are the most relevant categories of environmental law and policy.[39] Multilateral environmental agreements (MEAs) are often negotiated in the shape of framework conventions characterized by the comparatively general character of the obligations codified therein, which takes into account existing conflicts of interests of the Contracting Parties.[40] These conflicts arise from the fact that environmental regulation

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[34] *Mallet*, Nature 446.7133 (2007): 279-283, 279.

[35] *Lorenzini et al.,* Wolf–dog crossbreeding: "Smelling" a hybrid may not be easy. Mammalian Biology - Zeitschrift für Säugetierkunde 79.2 (2014): 149-156, 154.

[36] *Ibid.*, 154-155.

[37] *Proelß* , Die Umwelt im Völkerrecht , in *Graf Vitzthum/Proelß*, Völkerrecht (2013): 403–444, 403.

[38] *See* A. Factual Background, Anthropogenic causes for hybridization.

[39] *Epiney/ Scheyli*, Umweltvölkerrecht, Stämpfis juristische Lehrbücher (2000): 34-43, 34 *et seq*.

[40] *Ibid.*, 35-37.

usually impacts other political sectors such as trade and economy.[41] Different to MEAs soft law consists of non-binding resolutions and declarations which are recommended for implementation by States parties to a binding international agreement or an international organization (IO).[42] Notwithstanding its non-binding character, soft law has an enormous impact on international environmental policy as it represents a "precursor"[43] for legally binding environmental regulations. One example for such a powerful soft law tool is the concept of sustainable development. This concept was first developed in the report "Our Common Future" of the World Commission on Environment and Development (WCED) in 1987[44] and then included in the (equally non-binding) Rio Declaration[45] with particular regard to the needs of future generations. Other important functions of soft law are pointed out by Alan Boyle[46]:

> […] soft law instruments are used as mechanism for authoritative interpretation or amplification of the terms of a treaty.
> […] provide the detailed rules and technical standards required for implementation of some treaties.
> […] Some treaty give binding force to soft- law instruments by incorporating them into the terms of a treaty by implied reference. […].

It can thus be concluded that apart from treaties, soft law is an essential instrument of international environmental law and policy to realize the goal of sustainable use of natural resources, and to reduce the impact of human activities on the environment respectively.

All that said, it is important to note that the objectives of international environmental policy can only be reached if measures are taken into account before a threat to the environment emerges from human activities, or if it is eliminated directly after it occurs. Considering that environmental processes such as hybridization which negatively affect species, biodiversity and human well-being, are often characterized by a significant degree of scientific uncertainty, one of the most important principles of international

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[41] *Ibid.*, 17-33, 30. *Proelß* , Die Umwelt im Völkerrecht , in *Graf Vitzthum/Proelß*, Völkerrecht (2013) 403–444, 403-404.

[42] *Ibid.*, 34-43, 42. *Epiney/Scheyli*, Strukturprinzipien des Umweltvölkerrechts, Forum Umweltrecht (Baden-Baden: Nomos 1998) Vol. 29, 76-84, 77-78.

[43] *Epiney/ Scheyli*, Strukturprinzipien des Umweltvölkerrechts, Forum Umweltrecht (Baden-Baden: Nomos 1998) Vol. 29, 76-84, 80.

[44] WCED, Our Common Future 1987, transmitted to the General Assembly as an Annex to document A/42/427 – Development and International Co-operation.

[45] Rio Declaration on Environment and Development, *United Nations (UN)*, UN Doc. A/CONF.151/26 (Vol. I); 31 ILM 874 (1992) (Rio Declaration)

[46] *Boyle*, Some reflections on the relationship of Treaties and Soft Law, International and Comparative Law Quarterly, Vol. 48, 901-913, 905-906.

environmental law is the precautionary principle. This principle represents a strategic instrument for implementing the objectives of the concept of sustainable development.[47] Principle 15 of the Rio Declaration is generally regarded as containing the minimum standards applicable within its scope:

> In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.

The precautionary principle is thus applicable in situations characterized by scientific uncertainty, and where a high possibility of environmental damage exists.[48] Due to the fact that it has been included in numerous international treaties, the precautionary principle may be considered as being valid also under general customary international law.[49] That said, taking into account that the framing and scope of that principle differ among treaties, a general obligation (i.e. an obligation that applies irrespective of individual treaties) of States to take conservation-oriented measures whenever there is a possible environmental risk, does not seem to exist.[50] The fact that the exact scope of the precautionary principle depends on the individual situation of the case at hand leads to a substantial legal uncertainty. Therefore, it must be analyzed which role it plays in the context of hybridization.

As stated above, hybridization can be a threat for populations of species which often results from anthropogenic activities. However, the real extent of anthropogenic hybridization processes in the animal world is unknown, and so is the extent of consequences.[51] The more important it is to assess whether and, in the affirmative, to what extent international environmental law has recognized hybridization as a regulatory challenge.

The three most important international conventions in this context are the Convention on International Trade of Endangered Species of Wild Flora and Fauna

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[47] *Epiney/ Scheyli*, Umweltvölkerrecht, Stämpfis juristische Lehrbücher (2000) 75-100, 87-88.

[48] *Ibid.*, 86-87.

[49] *Proelß* , Die Umwelt im Völkerrecht , in *Graf Vitzthum/Proelß*, Völkerrecht (2013) 411–416, 415; *Birnie/Boyle/Redgewell*, International Law and the Environment (2009), 160 *et seq*.

[50] *Ibid.*

[51] *Allendorf/ Leary/ Spruell/ Wenburg*, Trends in Ecology & Evolution 16.11 (2001): 613-622, 618; *Kelly/ Whiteley/ Tallmon*, The Arctic melting pot. Nature 468.7326 (2010): 891. *Trouwborst*, Exploring the Legal Status of Wolf-Dog Hybrids and Other Dubious Animals: International and EU Law and the Wildlife Conservation Problem of Hybridization with Domestic and Alien Species, RECIEL 23.1 (2014): 111-124.

(CITES), the Convention on Biological Diversity (CBD) and the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). These conventions are MEAs which create binding international obligations vis-à-vis their parties.[52] All three of them refer to the precautionary principle, be it in non-legally binding resolutions, decisions, or preamble texts. In 2013, the States parties to CITES adopted Resolution Conference 9.24 (Rev. CoP16)[53]:

> […] RESOLVES that, by virtue of the precautionary approach and in case of uncertainty regarding the status of a species or the impact of trade on the conservation of a species, the Parties shall act in the best interest of the conservation of the species concerned and, when considering proposals to amend Appendix I or II, adopt measures that are proportionate to the anticipated risks to the species; […].

With regard to the CBD, the Preamble text essentially repeats the codification of the precautionary principle contained in Principle 15 of the Rio Declaration:

> […] Noting also that where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat, […].

Furthermore, the precautionary approach has been included in numerous decisions and guidelines adopted by the Parties to the CBD which will be analyzed in more detail below. The Bern Convention[54] also refers to the precautionary approach in two recommendations on invasive species[55]:

> Desirous of laying down a minimum number of rules, accepted and applied by everyone, aimed at anticipating and repairing the damage caused by inopportune introductions and which should be based essentially on principles of precaution and prevention, and referring to the "polluter-pays" principle; [56]

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[52] *United Nations Environment Programme* (UNEP) developed on the Stockholm Conference 1972; A/RES/27/2997. *See UNEP* Guides for Negotiators of Multilateral Environmental Agreements, 7.

[53] *CITES* Resolution Conf. 9.24 (Rev. CoP16) (Bangkok, Thailand, 03-14 March 2013).

[54] *Convention on the Conservation of European Wildlife and Natural Habitats* (19 September 1979); in force 1st November 1983 (Bern Convention).

[55] *Bern Convention, Standing Committee* Recommendation No. 57 (1997) on the introduction of organisms belonging to non-native species into the Environment (Adopted by the Standing Committee on 5 December 1997); Recommendation No. 77 (1999) on the eradication of non-native terrestrial vertebrates, adopted by the Standing Committee on 3 December 1999).

[56] *Bern Convention, Standing Committee* Recommendation No. 57 (1997).

In the following sections the general legal obligations contained in the three conventions are discussed. The main aim, though, is to point out whether hybridization and hybrids have been addressed by the conventions.

II. The Convention on the International Trade of Endangered Species of Wild Flora and Fauna (CITES)

(1) General Information on the Convention

The Convention on the International Trade of Endangered Species of Wild Flora and Fauna[57] was drafted in the course of the 8th meeting of the General Assembly of the International Union for Conservation of Nature (IUCN)[58] in 1963. The IUCN draft constituted the basis for a further recommendation of the UNCHE[59] in 1972, which then resulted in the adoption of CITES in 1973 with the aim to "ensure that international trade in specimens of wild animals and plants does not threaten their survival". Historically, CITES is one of the first products of "the modern era"[60] of international environmental law. It regulates trade with about 5,600 animal and about 30,000 plant species, subspecies and single populations of species.[61] CITES pursues a convention and annexes approach, in which endangered species are listed in different appendices to CITES depending on how threatened a species is by international trade.[62] Such an approach represents an effective measure as Article XV determined a simplified procedure for the amendments to Appendices I and II.[63] Furthermore, the legal consequences vary strongly depending on the

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[57] *Convention on International Trade of Endangered Species and of Wild Flora and Fauna* (CITES) (USA, Washington D.C, 3 March 1973); in force 1 July 1975. 180 countries joined CITES (June 2014). CITES represents an international treaty law which is approved by the member states (called "States parties") with the voluntary accession and has to be implemented into national law.

[58] *International Union for Conservation of Nature and Natural Resources* (IUCN) founded at 5th October 1948, (Fontainebleau, France) is a nongovernmental organization with the aim "to influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable", http://www.iucn.org/about/, last accessed August 2014).

[59] *United Nations Conference on Human Environment* (UNCHE), Stockholm Conference, 5 -6 July 1972.

[60] *Sand,* The Evolution of International Environmental Law, in *Bodansky/ Brunnée/ Hey,* The Oxford Handbook of International Environmental Law (2007): 31–43, 33.

[61] Effective October 2013.

[62] *CITES* Article II (1-4); Article III-V. In Appendix I species threatened with extinction are listed. Trade is only permitted in exceptional cases and with special import and export permits or re-export certificates. In Appendix II species are listed whose trade must be controlled to avoid overexploitation. These species are not necessarily threatened with extinction, however, trade is only permitted with special export permits. In case a Member State requests other States Parties to CITES for support in controlling trade of a species only protected under the respective national law, this species is listed in Appendix III. Trade is only permitted with appropriate permits and certificates.

[63] *CITES* Article XV. Amendments to Appendices I and II are discussed at the meeting of the Conference of Parties (CoP) and shall be adopted by a two-thirds majority of States parties. Amendments adopted on the

appendix a species is listed in. In Appendix I species threatened with extinction are listed and commercial trade is generally prohibited. Only in exceptional cases (i.e. scientific and educational purposes or hunting trophies) trade is permitted with special import and export permits and/or re-export certificates. In Appendix II species are listed whose trade must be controlled according to predicted adverse impacts on their survival if trade is uncontrolled. Commercial trade of Appendix II species is permitted under certain conditions (i.e. special export permits). In case a Member State requests other States parties to CITES for support in controlling trade of a species only protected under the respective national law, this species is listed in Appendix III. Trade is only permitted with appropriate permits and certificates. All permits and certificates shall be granted only if a detrimental effect on the survival of the wild species by trade can be excluded by the Scientific Authority and/or the Management Authority.[64] In order to meet the requirements of this convention, a specific legal obligation of CITES is its implementation into domestic legislation by each States party.[65] The Conference of Parties (CoP) represents the decision-making body to CITES which is responsible for reviewing the implementation of the obligations contained in CITES.[66] In the Resolution Conference 8.4 (Rev. CoP15) the CoP to CITES summarized four basic measures, determined in Article VIII of the convention that shall be implemented by the States parties:

a. designate the Scientific and Management Authorities,
b. prohibit the trade in specimens in violation of the convention
c. penalize trade in violation of the convention, and
d. confiscate specimens illegally traded or possessed.[67]

The CITES administrative part is the CITES Secretariat which is provided by the Executive Director of the UNEP.[68] The Standing Committee oversees the management of the Secretariat and gives guidance to the implementation of the convention.[69] The Animals Committee was established together with the Plant Committee at CoP 6 (Ottawa, 1987) to

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CoP shall enter into force 90 days after the meeting of CoP; *Proelß* , Die Umwelt im Völkerrecht, in *Graf Vitzthum/Proelß*, Völkerrecht (2013) , 403- 444, 420.

[64] *CITES*, Article VI; Article IX.

[65] *CITES*, Article VIII.

[66] *CITES*, Article XI, XI.3 *et seq.*

[67] *CITES*, Article VIII.1. *CITES* Resolution Conf. 8.4 (Rev. CoP15) amended at the 14th and 15th Meeting of the CoP (Doha, Qatar, 13-25 March 2010), the CoP to the Convention on the National laws for implementation of the Convention.

[68] *CITES*, Article XII.1.

[69] *CITES* Resolution Conf. 11.1 (Rev. CoP16) amended at the 12th, 13th, 14th and 16th Meeting of the CoP (Gigiri, Kenya, 10-20 April 2000), the CoP to the Convention on the Establishment of committees, Annex 1.

give biological and technical advice to decision makers.[70] In order to implement the provisions of the convention in a harmonized way, the often broad provisions are supplemented by an agreement that serves as guidance for implementation.[71] Therefore, non-legally binding instruments such as resolutions or decisions, provided by the CoP, are of particular importance as guidance documents to improve the effectiveness of the convention.[72] The States parties to CITES adopt resolutions and decisions by consensus or two-thirds majority at the respective meetings.[73] Resolutions are long-standing guidance documents such as documents on the interpretation of the convention or the establishing of the permanent committees. By contrast, decisions, in general, contain instructions for specific committees or the secretariat which often have to be implemented at a specified time limit.[74] Although CITES represents an international treaty law which is legally binding for its States parties[75], the implementation of CITES varies.[76] Article VIII of the convention provides *inter alia*:

> 1. The Parties shall take **appropriate** measures to enforce the provisions of the present Convention and to prohibit trade in specimens in violation thereof. These shall include measures:
> (a) to penalize trade in, or possession of, such specimens, or both; and
> (b) to provide for the confiscation or return to the State of export of such specimens.

Reasons for the different degree of implementation are for example the provisions in Article VII and XXIII offering, depending on the circumstances, the opportunity to essentially ignore some obligations.[77] The ratio of these provisions was to introduce the necessary degree of flexibility. However, they were used by some States as a loophole for weaker implementation.[78] Other authors referred to "(i) financial, (ii) technical, (iii)

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[70] *Ibid*., Annex 2.

[71] *CITES,* Article XI.3.

[72] *Epiney/ Scheyli*, Umweltvölkerrecht, Stämpfis juristische Lehrbücher (2000) 34-43, 41. Article XI.3 *CITES*.

[73] *CITES* Resolution, Decision (2015), *CITES* Rules of Procedure of the Conference of the Parties adopted at CoP16 (Bangkok, Thailand, 03-14 March 2013), http://www.cites.org, last accessed January 2015).

[74] *Ibid.*

[75] Convention on the Law of Treaties (VCTL, Vienna) of 25 May 1969, in force 27 January 1980, 1155 U.N.T.S. 331, 8 I.L.M. 679, Article 26.

[76] *Reeve*, Policing International Trade in Endangered Species: The CITES Treaty and Compliance, The Royal Institute of International Affairs (London, 2002): 35-48, 35.

[77] *Ibid.*, 35.

[78] *Ibid.*, 35; However, within the Resolution Conf. 11.3 (Rev. CoP16) and 8.4 (Rev. CoP15) the CoP to CITES recognizes the problem and seeks to improve the compliance and enforcement of the provisions and calls, therefore, for the implementation into national law by the States parties. *CITES* Resolution Conf. 11.3 (Rev. CoP16), the CoP to the Convention on Compliance and Enforcement (Bangkok, Thailand, 03-14

scientific, (iv) legal and administrative and (v) political"[79] obstacles as rationale for the "non-compliance response"[80] to the convention.

In order to improve compliance CITES has evolved a compliance system through secondary rules (resolutions and decision) and practice over the last decades which include technical assistance, workshops and ad hoc Secretariat missions.[81] Furthermore, the States parties are required to prepare annual trade reports and biennial implementation reports.[82] The CITES Secretariat monitor and report on compliance to the CITES Standing Committee and/or the CoP. Non-compliance with these obligations constitutes a violation of CITES and could involve legal consequences for the States party concerned. In this case, the CITES Standing Committee may impose sanctions against the States party by recommending trade suspensions of CITES-listed species.[83] In general such trade sanctions will be lifted after compliance requirements are fulfilled by the States party concerned.

(2) Hybridization in the context of CITES

There are two resolutions that address hybrids and one dealing with hybridization that were adopted by the States parties to CITES. Due to the fact that hybridization can arise from introducing invasive species in a non-native habitat, a convention regulating trade in species has the potential to play an important role in setting conservation guidelines for the treatment of hybrids. The term 'hybrid' is defined in the glossary of CITES as:

> an animal or plant that is produced as the result of cross-breeding between two different species.[84]

The glossary which is a collection of terms used by the text of the convention or that of resolutions, does not contain a separate definition of the term 'hybridization'.

March 2013); *CITES* Resolution Conf. 8.4 (Rev. CoP15), the CoP to the Convention on National laws for implementation of the Convention. (Doha, Qatar, 13-25 March 2010).

[79] *Bowman/ Davies/ Redgewell*, Lyster`s International Wildlife Law (ed. 2; 2010), Cambridge University Press 92-118, 113.

[80] *Reeve*, Policing International Trade in Endangered Species: The CITES Treaty and Compliance, The Royal Institute of International Affairs (London, 2002), 91.

[81] *Reeve*, Wildlife trade, sanctions and compliance: lessons from the CITES regime, International Affairs (2006) 82.5: 881-897, 881 and 885; *CITES* Resolution Conf. 14.3, the CoP to the Convention on CITES compliance procedures. (the Hague, Netherlands, 03-15 June 2007), 4.

[82] *Ibid*., 884; *CITES*, Article VIII (7).

[83] *Reeve*, Wildlife trade, sanctions and compliance: lessons from the CITES regime, International Affairs, Blackwell Publishing Ltd (2006) 82.5: 881-897, 886- 887.

[84] *CITES* glossary; *IUCN* glossary: Hybridization is defined as a cross-breeding between individuals of different species.

The issue of hybridization has found its way into the CITES regime several years before it has become an issue in other international fora. The term ‘hybrid’ appeared for the first time in the second meeting of CITES (San José, 1979) in Resolution Conf. 2.13 on the “Problem of Hybrids” stating that:

> […] the fundamental purpose of the Convention is to conserve wild fauna and flora as part of the natural system of the earth;
> RECOGNIZING also that many hybrids are transient in occurrence and may work to diminish the genetic integrity of taxa through introgression of the gene pools of parental populations; […].

The main aim of this resolution, though, was to improve the effectiveness of the convention by including a resolution on hybrids in order to ensure an enhanced protection of the listed species. The identification of species or hybrids may be insufficient or detective which may lead to a detrimental effect on the protection of listed species if trade is uncontrolled.[85] Consequently, the Conference of Parties (CoP) to CITES decided in the same Resolution in 1979 (Resolution Conf. 2.13):

> […] a) that hybrids may be specifically included in the Convention appendices, but shall be included only if they form distinctive and stable populations in the wild;
> b) that hybrids are subject to the provisions of the Convention even though not specifically included in the appendices if one or both of their parents are of taxa included in the appendices;
> c) that if the parents of a hybrid specimen are included in different appendices, the provisions of the more restrictive appendix shall apply; and
> d) that determinations whether trade in unlisted hybrids will not be detrimental to survival shall be made with reference to survival of the included parental taxa of to survival of other taxa that were meant to be protected by inclusion of the parental taxa. […].

The first provision (a) enables the inclusion of hybrids in the appendices to CITES if they form separate and distinct populations, e.g. because of a better adaptation to current environmental conditions compared to their parental species. The adaptation to new living conditions as well as the evolution of new species is necessary to ensure biodiversity in the future. In accordance with this aim, such a provision is clearly useful in order to preserve

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[85] *CITES* Resolution Conf. 2.13, the CoP to the Convention on the Problems of Hybrids (San José, Costa Rica, 19-30 March 1979).

natural hybrid populations and to avoid an immediate disturbance by trade. While the provisions for species listed in one of the appendices[86] also prevent illegal trade, a provision specifically applicable to hybrids is necessary, as trading entities could otherwise circumvent these regulations by labeling pure species as hybrids if they are morphologically indistinguishable. Especially hybrids of domesticated species and their wild relatives could arouse the interest of trading entities and animal owners. Thus, the risk of false labeling would potentially increase without a regulation on hybrids. Based on the provisions (b) and (c) it can be assumed that only first generation hybrids or backcrosses (hybrid x parental species) are within the scope of this resolution as at least one parental species listed in the appendices have to be in their lineage. Therefore, trade of second or further generation hybrids remains uncontrolled. However, at this time this resolution only refers to hybrids in question of their trade. The role of trade as potential driver of biological invasion (i.e. escaped or suspended non-native species) which in turn may lead to hybridization processes in the wild is not considered in this resolution or other relevant documents. Almost twenty years later, the States parties to CITES discussed the applicability and usefulness of Resolution Conf. 2.13.[87] The Chairman critically referred to the administrative efforts associated with a strict implementation of the Resolution Conf. 2.13 if captive-bred hybrids were still included. The Secretariat agreed that in this case Resolution Conf. 2.13 could be contrary to the general principles of the convention to facilitate the trade in captive-bred species. The US observer criticized that without Resolution Conf. 2.13, a gap in the law would occur, as trading entities would be tempted to classify purebred specimen as hybrids to evade sharper controls.[88] Hybrids are not always distinguishable based on morphological characteristics and even genetic methods are not always accurate, which is why the objection raised by the US observer was clearly justified. Especially for species threatened with extinction with regard to which trade is only permitted in exceptional cases, the consequences of such an illegal trade could be dramatic. Hence, at the 13th meeting of the Animals Committee a new draft resolution was elaborated by the Secretariat, the Chairman and the US observer to replace Resolution Conf. 2.13.[89] At the 10th meeting of the CoP (Harare, June 1997) Resolution Conf. 10.17 on animal hybrids was adopted which replaced Resolution Conf. 2.13. This resolution

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[86] Article III-V, *CITES*. A licensing system of import, export and re-export permits for species listed in one of the annexes regulates their trade.

[87] *CITES* AC 12 Summary record, 12th Meeting of the Animals Committee (AC) (Antigua, Guatemala, 11-14 Sept. 1995), (10), 14.

[88] *Ibid*., 14. *CITES* Agenda & Working Documents; Agenda XIV. 36a, b, Doc. 10.70 of the 10th Meeting of CoP (Harare, Zimbabwe, 9-20 June 1997), 843.

[89] *Ibid*., 843.

outlined that hybrid animals should be treated as parental species if at least one is included in the appendices. However, its scope is limited to hybrid animals which have such a parental species in their "recent lineage",[90] i.e., in their previous four generations.[91] Since that time, however, the CoP changed the content of this resolution. At CoP 14, article a)[92] of the resolution was transferred to Resolution Conf. 9.24 (Rev. CoP16) on the Criteria for amendment of Appendices I and II[93]:

> g) hybrids may be specifically included in the Appendices but only if they form distinct and stable populations in the wild. […]

It can thus be concluded that CITES has ensured that the precautionary approach is applied by regulating the trade of hybrids descending from at least one listed species considering the uncertainty "regarding the status of a species or the impact of trade on the conservation of a species"[94] (i.e. in distinguishing hybrids from their purebred relatives). Nonetheless, it is a non-legally binding resolution and the same applies in this case to the application and implementation of the precautionary approach by the States parties.

The States parties to CITES have not only taken into account the role of hybrids in questions of trade. Also the possible threat to populations due to hybridization in the wild was mentioned in the annexes to Resolution Conf. 10.7 (Rev. CoP15) on the "Disposal of confiscated live specimens of species included in the Appendices".[95] The "Guidelines for the disposal of Confiscated Animals" and "Guidelines for Re-introductions" developed by the IUCN were thereby introduced into the annexes of the aforementioned Resolution.[96] In Annex 1 the "Statement of need" pointed out that an unregulated release of species could lead to the risk of interspecific hybridization which was described in more detail in the

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[90] *CITES* Resolution Conf. 10.17 (Rev. CoP14) (The Hague, Netherlands, 03-15 June 2007), the CoP to the Convention on Animal Hybrids.

[91] *Ibid., d) as a guideline, the words "recent lineage", as used in this Resolution, shall generally be interpreted to refer to the previous four generations of the lineage.*

[92] *CITES* Resolution Conf. 10.17 before CoP14: *a) that hybrids may be specifically included in the Convention appendices, but shall be included only if they form distinctive and stable populations in the wild*.

[93] *CITES* Resolution Conf. 9.24 (Rev. CoP16) (Bangkok, Thailand, 03-14 March 2013), the CoP to the Convention on the Criteria for amendment of Appendices I and II.

[94] *CITES* Resolution Conf. 9.24 (Rev. CoP16) (Bangkok, Thailand, 03-14 March 2013). "RESOLVES that, by virtue of the precautionary approach and in case of uncertainty regarding the status of a species or the impact of trade on the conservation of a species, the Parties shall act in the best interest of the conservation of the species concerned and, when considering proposals to amend Appendix I or II, adopt measures that are proportionate to the anticipated risks to the species".

[95] *CITES* Resolution Conf. 10.7 (Rev. CoP15) (Doha, Qatar, 13-25 March 2010), the CoP of the Convention on the Disposal of confiscated live specimens of species included in the Appendices; Annex I, 3.

[96] *Ibid.*, 1. *See also IUCN* "Guidelines for the Placement of confiscated animals" approved at the 51th Meeting of the IUCN council (Gland, Switzerland, Feb. 2000).

section “Decision Tree Analysis- Return to the wild”:

> If the country of origin and site of capture of the animals are not known, release for reinforcement may lead to inadvertent **hybridization** of distinct genetic races or subspecies resulting in outbreeding depression. Related species of animals that may live in sympatry in the wild and never hybridize have been known to hybridize when held in captivity or shipped in multi-species groups. This type of ‘mis-imprinting’ can result in behavioural problems compromising the success of any future release and can also pose a threat to wild populations by artificially destroying reproductive isolation that is behaviourally controlled.[97]

CITES recognized that even the release in alleged native habitats could cause hybridization by breaking up reproductive isolation. Thirty years after the first resolution on animal hybrids was adopted and ten years after the IUCN published these guidelines, CITES adopted guidelines dealing with confiscated animals, which also consider the risk of hybridization in the wild. This long period illustrates that hybridization as a threat has been underestimated by conservationists for a long time and that it gains attention by decision makers only slowly. However, these guidelines represent a first step in ensuring the application of the precautionary approach in cases of the somewhat unpredictable consequences of the unregulated release of confiscated species (i.e. hybridization).

Currently, the treatment of hybrids and their conservation status is only regulated in a non-legally binding context of trade by CITES. Although hybridization is recognized as a threat, a specific provision in form of a resolution or decision to avoid hybridization with invasive species does not exist. Taking into account that CITES represents an international trade treaty to protect native species against overexploitation through international trade it appears natural that the ecological consequences of hybridization for biodiversity in general attracted only little attention. Nonetheless, the aforementioned guidelines illustrate that it is at least necessary to regulate the disposal or re-introduction of confiscated animals to prevent adverse effects such as hybridization.

III. The Convention on Biological Diversity (CDB)

(1) General Information on the Convention

The first international convention that relates to biodiversity in a comprehensive manner is

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[97] *CITES* Resolution Conf. 10.7 (Rev. CoP15), Annex I, “Decision Tree Analysis- return to the Wild, Question 3: Can country of origin and site of capture be determined?” 10.

the Convention on Biological Diversity (CBD).[98] The convention was adopted in 1992 at the Conference of the UNCED (Rio Conference) and entered into force on 29th December 1993.[99] The Rio Conference represented the next step in international environmental law after the Stockholm Conference and defined the start of the "post-modern era" which played an important role in developing international environmental policy.[100] The CBD is a multilateral treaty which is legally binding for its Parties.[101] It was developed to ensure the "conservation of biological diversity, the sustainable use of the components of biological biodiversity and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources".[102] Therefore, the CBD presented a new type of international agreement geared towards whole biodiversity conservation with an intrinsic approach.[103] The Conference of Parties (COP)[104] presents the decision-making body of the CBD which is supported by the Subsidiary Body on Scientific, Technical, and Technological Advice (SBSTTA).[105]

The CBD is more of a "soft formulated" legally binding law than a strict treaty.[106] This "soft character" has also led to an incomplete implementation of the essential standards by the Parties and is mainly due to conflicts of interest and territorial sovereignty.[107] Nevertheless, the CBD is an important international instrument for the sustainable use of natural resources and the protection of biodiversity with ambitious, however, non-legally binding targets for 2020 (Aichi targets). In 2010, the Parties approved a strategic plan on Biodiversity including five strategic goals and twenty targets.[108] The Aichi targets should provide a flexible, however, non-legally binding

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[98] *Convention on Biological Diversity* (CBD) opened for sign in 5th June 1992; into force 29 December 1993. 194 countries ratified the treaty, except the USA which only signed in 1993 (July 2014). Members are called Parties. *See also* http://www.cbd.int/information/parties.shtml. Administered under UNEP.

[99] *United Nations Conference on Environment and Development* (UNCED) (Rio Conference); (Brazil, Rio de Janeiro, 3- 14 June 1992).

[100] *Sand,* The Evolution of International Environmental Law, in *Bodansky/ Brunnée/ Hey,* The Oxford handbook of international environmental law (2007) 31–43, 33.

[101] Article 26, *VCTL.*

[102] http://www.cbd.int/intro/, last accessed August 2014).

[103] *CBD* Preamble; *Proelß* , Die Umwelt im Völkerrecht, in *Graf Vitzthum/Proelß,* Völkerrecht (2013) , 403-444, 435.

[104] Article 23, *CBD.*

[105] Ibid., Article 25. The SBSTTA is a committee composed of experts to support the Conference of Parties (COP).

[106] *Epiney/ Scheyli,* Umweltvölkerrecht, Stämpfis juristische Lehrbücher (2000) 41. *For example* the legal scope of the stipulated environmental impact assessments (EIAs) in Article 14 of the convention is weakened with the addition "where appropriate and allow" which allows the parties to act on its own responsibility and estimation. 287-288.

[107] *Proelß* , Die Umwelt im Völkerrecht, in *Graf Vitzthum/Proelß,* Völkerrecht (2013) , 403- 444, 436-437.

[108] *Ibid*., 437. UNEP/CBD/COP/DEC/X/2 on Strategic Plan for Biodiversity 2011-2020, *CBD,* (Nagoya, Japan, 18-29 October 2010).

framework for national and regional objectives. Three of five strategic goals (A, B, E)[109] are of particularly great interest in the context of hybridization. Addressing the value of biodiversity and the causes for biodiversity loss (Strategic Goal A) is the basis for a successful conservation strategy, which needs to be implemented by a wide collaboration using an improved knowledge (Strategic Goal E, Target 19).[110] In terms of hybridization, this improvement of scientific knowledge is certainly required. With the aim of reducing habitat loss, invasive species and multiple anthropogenic pressures on sensitive ecosystems (Target: 5, 9 and 10)[111] the Strategic Goal B comprises ambitious aims, which also include indirectly the aim of reduction of hybridization.

(2) Hybridization in the context of CBD

With regard to the scope of this study, the role of hybridization in reaching the Aichi Targets is particularly interesting. Although the terms 'hybridization' and 'hybrid' are used by the CBD in non-legally binding decisions and other non-legally binding documents, for example of the SBSTTA, no definition is proposed in any corresponding document or in the glossary of terms used by the CBD. Thus, the following analysis refers to the non-binding definition given by CITES.

In the context of the CBD, hybridization is not treated separately as a threat to biodiversity. However, factors that may trigger hybridization such as habitat change, climate change and particularly invasive species are outlined as major threats to biodiversity by the CBD.[112] The CBD calls for precautionary measures to prevent negative consequences of the infiltration of invasive species[113], habitat fragmentation[114] and even

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[109] *Ibid.*, IV. Strategic Goals and the Aichi Biodiversity Targets. "**A**. Address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society. **B**. Reduce the direct pressure on biodiversity and promote sustainable use. […] **E**. Enhance implementation through participatory planning, knowledge management and capacity-building."

[110] *Ibid.*, Strategic Goal **E**, "Target **19**: By 2020, knowledge, the science base and technologies relating to biodiversity, its values, functioning, status and trends, and the consequences of its loss, are improved, widely shared and transferred, and applied."

[111] *Ibid.*, Strategic Goal **B**, "Target **5**: By 2020, the rate of loss of all natural habitats, including forests, is at least halved and where feasible brought close to zero, and degradation and fragmentation is significantly reduced. Target **9**: By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment. Target **10**: By 2015, the multiple anthropogenic pressures on coral reefs, and other vulnerable ecosystems impacted by climate change or ocean acidification are minimized, so as to maintain their integrity and functioning."

[112] *Secretariat of the Convention on Biological Diversity* (2014) *Global Biodiversity Outlook 4*. Montréal, 24-25.

[113] UNEP/CBD/COP/DEC/XI/28 on Invasive Alien Species, *CBD*, **(**Hyderabad, India, 8-19 October 2012).

[114] UNEP/CBD/COP/DEC/X/2: Aichi Biodiversity Target 5, Strategic Goal **B**.

climate change[115] which in turn represent also precautionary measures to prevent hybridization processes.

However, hybridization is addressed in a few documents referring to specific thematic issues of the CBD.[116] The first thematic issue including hybridization is the cross-cutting issue of Invasive Alien Species (IAS).[117] The term 'hybridization' occurs in two documents on IAS published by the SBSTTA both of which point out the risk of hybridization for wild native populations.[118] At the same time, the SBSTTA calls for an increased research on the impact of invasive species, *inter alia*, on genetic levels "in order to develop an adequate knowledge base."[119] On the following COP the Parties to the CBD act on requests made by the SBSTTA and calls on the Parties to intensify their efforts in the field of research as set out in the corresponding decision.[120] Although the risk of hybridization was recognized by the SBSTTA, this term was not included in the aforementioned decision. This suggests that at this time the state of knowledge was not sufficient to consider hybridization a serious threat. In 2004, during COP 7 the Parties to the CBD noticed that legal uncertainties in the international regulatory framework exist regarding the definition of IAS and that released, bait or escaped specimens also could represent IAS.[121] In 2011, a "decision on invasive species introduced as animal pets, as aquarium and terrarium species and as live bait and live food" was adopted by the Parties

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[115] UNEP/CBD/COP/DEC/XI/21 on Biodiversity and climate change: integrating biodiversity considerations into climate-change related activities, *CBD,* (Hyderabad, India, 8-19 October 2012).

[116] Taking into account the complexity of biodiversity, the COP established thematic programs of work and cross-cutting issues with the aim to develop and adopt (concrete) issue-specific guidelines and/or principles to improve the effectiveness of this Convention, http://www.cbd.int/programmes/, last accessed March 2015.

[117] *CBD*- glossary: Definition of alien species: "A species, subspecies or lower taxon, introduced outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce". Definition of invasive alien species: "An alien species whose introduction and/or spread threaten biological diversity", http://www.cbd.int/invasive/terms, last accessed September 2014.

[118] UNEP/CBD/SBSTTA/6/7 on Invasive species -Comprehensive review on the efficiency and efficacy of existing measures for their prevention, early detection, eradication and control, *SBSTTA*, (Montreal, Canada 12-16 March 2001), Annex: Adverse impacts of invasive alien species, 87: "(b) Hybridization between alien and related native species, including wild relatives. […] Hybridization can threaten a native species even when the hybrids do not succeed, simply because crossbreeding reduce the number of new offspring added to the species' own population. Hybridization can also produce in some cases new invasive species; and […]"; *see also* UNEP/CBD/SBSTTA/6/INF/11, on Invasive species, *SBSTTA,* (Montreal, Canada 1-10 March 2001), 7; *see also* UNEP/CBD/SBSTTA/6/6, on Invasive species- *Progress report on matters identified in decision V/5, paragraphs 5, 11 and 14, and an analysis of national reports* III. Review of case-studies, *SBSTTA,* (Montreal, Canada 12-16 March 2001), F. General points/issues 39, 10.

[119] UNEP/CBD/SBSTTA/REC/VI/4 on Alien species that threaten ecosystems, habitats or species, *SBSTTA,* (Montreal, Canada 12-16 March 2001): Annex Guiding principles for the prevention, introduction and mitigation of impacts of alien species that threaten ecosystems, habitats or species. Guiding principle 5.

[120] UNEP/CBD/COP/DEC/VI/23 on Alien species that threaten ecosystems, habitats or species, *CBD,* (The Hague, Netherlands, 7-19 April 2002), Guideline principle 5.

[121] UNEP/CBD/COP/DEC/VII/13, 227- 230, Alien species that threaten ecosystems, habitats or species (Article 8 (h)), *CBD* (Malaysia, Kuala Lumpur, 9 - 20 February 2004), 228.

to the CBD.[122] Furthermore, it was recognized that escaped animals from zoo populations could pose a threat as invasive alien species (COP/DEC/XI/28/8). Additionally, the COP (DEC/XI/28/8) was concerned about:

> [8. *Concerned* about] … the potential risks associated with the intentional and unintentional release or escape of individuals of captive-bred alien populations and genotypes of pets, aquarium and terrarium species, or species used as live bait and live food, which may have an impact on **native genetic diversity**, and *noting* the need to document these and develop guidance on how to deal with them, *requests* the Executive Secretary to collect case studies and to explore measures, in collaboration with relevant international organizations, on how to deal with such risks;

Although hybridization is not explicitly mentioned in this section, "the impact on native genetic diversity" by invasive alien species including domesticated and captive-bred animals obviously refers to hybridization. It could be interpreted as a call for increased research efforts concerning the risk of hybridization and how to deal with escaped and released alien species or their hybrids. Three years later the SBSTTA published a corresponding Note including draft guidance "on devising and implementing national measures to address the risks associated with the introduction of alien species […]".[123] In this context the term 'hybrid' appeared for the first time in a CBD document:

> 53. For the purpose of this guidance, pets, aquarium and terrarium species, live bait and live food are understood to include lower taxa and hybrids (including hybrids between native organisms and organisms that are alien in the region to which they are intended to be imported or transported).[124]

Within this draft guidance hybrids obtain the same status and recommendation for treatment as their purebred invasive relatives. This appears quite reasonable considering that negative effects of invasive species are likely to be very similar for hybrids, and that particularly the risk for wild native species to hybridize may even increase for hybrids compared to purebred invasive species. Furthermore, it is required under guiding principle 2 of this document that:

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[122] UNEP/CBD/COP/DEC/XI/28 on Invasive alien species, *CBD*, (Hyderabad, India, 8-19 October 2012).

[123] UNEP/CBD/SBSTTA/18/8 on Management of risks associated with introduction of alien species as pets, aquarium and terrarium species, and as live bait and live food, and related issues, *SBSTTA*, (Montreal, Canada 23-28 June 2014).

[124] *Ibid.*, 11.

> […] Thus in the case that invasive species have escaped or have been released and become established, measures for early detection and rapid response, including eradication, should be undertaken; and where eradication is not feasible, containment and long-term control measures should be applied.[125]

Considering the aforementioned interpretation, this guiding principle also includes the eradication of introduced hybrids. The question arises how to deal with wild-born hybrids (resulting from hybridization with invasive species in the wild). Article 8 (h) of the CBD states:

> Each contracting Party **shall**, as far as possible and as appropriate, prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species.

In this context, it remains unclear if a wild-born hybrid of a wild native species and an invasive alien species falls within the scope of the aforementioned article. A legally or politically binding definition of hybrids or invasive species addressing this issue is not provided in any CBD document. Furthermore, if the native species involved is listed in the appendices to CITES, a conflict in terms of the conservation status of this species could arise. For example, species A is listed in Appendix I to CITES and thus obtains the maximal protection status under CITES (including its hybrids to the fourth generation). If this species A hybridizes with the invasive alien species B (not protected under CITES), the conservation status of the hybrid offspring depends on the underlying convention. Under CITES this hybrid would obtain the same protection as species A. Under the CBD, however, this hybrid would be a subject to control or eradicate if wild-born hybrids of native and invasive alien species would be included in the definition of invasive alien species. Thus, it can be concluded that, taking into account the potential negative impacts of hybridization on non-native species[126], a removal of invasive alien species and their hybrids constitutes an appropriate measure to ensure the survival of the native species. If the conservation status of hybrids presented by CITES would only refer to the trade of hybrids and not to the general conservation status of a hybrid, this conflict may be solved.

Furthermore, the aforementioned note of the SBSTTA contains an example of

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[125] *Ibid.*, 3.

[126] *Huxel*, Rapid displacement of native species by invasive species: effects of hybridization. Biological Conservation 89.2 (1999): 143-152, 143.

implementation for the import of live individuals of a species in Australia.[127] The Australian fauna is unique with a high proportion of endemic[128] species. In order to protect this unique fauna against invasive species, Australia implemented a general rule on the import of live individuals of non-native species into national law. Therefore, the import of hybrids is prohibited unless hybrid species are explicitly listed in the live import list provided by the Australian government.[129] Interestingly, the Australian national law, in contrast to CITES, does not differentiate between the generational distance from the wild ancestor or original interbreeding event.[130] This form of dealing with hybrids provides a strict example of controlling the introduction of invasive species by trade and may provide a reference for other countries. However, it is questionable if such a strict trade regulation is applicable to other nations since the trade and therefore a part of the economic sector would be strongly restricted. By derogation from such a strict regulation, a softer approach such as a general import prohibition for hybrids of threatened species may be more enforceable.

From the aforementioned note[131] a recommendation was worked out by the SBSTTA in 2014 recommending the COP at its twelfth meeting to adopt a decision towards “the management of risk associated with the introduction of alien species […]” including the aforementioned approaches concerning hybrids.[132] These approaches illustrate that hybridization and hybrids obtain increasing attention in the context of conservation.

With regard to the thematic issue of Island Biodiversity, the COP to the CBD already requested the development of science-based risk assessment methodologies including the risk of hybridization by the introduction of invasive species, which could also be implemented in other aspects of the CBD.[133] Similar to Australia, many islands are known to have unique endemic diversity of flora and fauna. The introduction of invasive

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[127] UNEP/CBD/SBSTTA/18/8, 6.

[128] Endemic species are species which only occur in particular definite areas.

[129] *Australian Government – Department of Environment,* Guidance on the import of live hybrid animals: “Currently, the live import list explicitly includes one hybrid (*Equus asinus x Equus caballus*, Mule/ Hinny) and specifically excludes one hybrid (the Savannah cat - a cross between a domestic cat and an African Serval)*”*, http://www.environment.gov.au/biodiversity/wildlife-trade/live/import-list/hybrid-animals, last accessed March 2015.

[130] *Ibid*.

[131] UNEP/CBD/SBSTTA/18/8.

[132] UNEP/CBD/SBSTTA/REC/XVIII/5, Recommendation on Management of risks associated with introduction of alien species as pets, aquarium and terrarium species, and as live bait and live food, and related issues, *SBSTTA* (Montreal, Canada 23-28 June 2014).

[133] UNEP/CBD/COP/DEC/VIII/1 on Island Biodiversity, *CBD*, (Curitiba, Brazil, 20-31 March 2006); Priority action 6.1.3.9; 27; “Develop science-based risk assessment methodologies applicable at the local, national and regional levels, including the risk of hybridization with endemic species. (D = working definition: Priority action = major action that must be implemented […])”.

species resulting, *inter alia*, in hybridization with native species is a major threat to those species. Considering the endemic status of a species on a particular island, its genetic displacement by hybridization would be equivalent to its complete extinction. Approaches as the aforementioned import provisions would minimize this risk.

However, the CBD does not only recognize the risk of hybridization in the context of biological invasions but also the interactions between climate change and biodiversity. The SBSTTA noticed that climate change affects species indirectly by affecting species interactions. As the real impact of climate change on biodiversity is difficult to assess[134], hybridization is not explicitly mentioned in a climate change related document of the CBD. Nevertheless, the COP requested in their Decision XI/21 in 2012 the continuation on gathering information and knowledge to integrate “biodiversity considerations into climate change related activities”, as recently recommended by the SBSTTA.[135] The scientific literature provides increasing evidence for the impacts of climate change on hybridization. In addition to range shifts and phenological shifts, physiological and behavioral changes have also been documented as potential effects of climate change, which in turn may facilitate hybridization.[136] Nevertheless, scientific uncertainty persists concerning the real extent of climate change and therefore its potential impact on hybridization. This confirms that, in accordance with the precautionary principle, hybridization facilitated by climate change and other anthropogenic factors should gain increased attention in future decisions of the CBD. Notwithstanding, the CBD calls upon the Parties to take precautionary measures in order to prevent negative consequences of the infiltration of invasive species[137], of habitat fragmentation[138] and even climate change[139], which in turn represents, even if not explicitly mentioned, precautionary measures in order to prevent hybridization processes.

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[134] UNEP/CBD/SBSTTA/14/INF/21, 13, Report of the second meeting of the Ad Hoc Technical Expert Group on Biodiversity and Climate Change, *SBSTTA*, (Nairobi, Kenya 10-21 March 2010).

[135] UNEP/CBD/COP/DEC/XI/21 on Biodiversity and climate change: integrating biodiversity considerations into climate-change-related activities, *CBD*, (Hyderabad, India, 8-19 October 2012); UNEP/CBD/SBSTTA/REC/XVI/8 Recommendation: Proposals on integrating biodiversity considerations into climate-change-related activities, including addressing gaps in knowledge and information, *SBSTTA*, (Montreal, Canada, 30 April - 5 May 2012).

[136] *Chunco*, Ecology and Evolution 4.10 (2014): 2019-2031.

[137] UNEP/CBD/COP/DEC/XI/28 on Invasive Alien Species, *CBD*, **(**Hyderabad, India, 8-19 October 2012).

[138] UNEP/CBD/COP/DEC/X/2 Aichi Biodiversity Target 5, Strategic Goal **B**, www.cbd.int/sp/targets/#GoalB, last accessed November 2014.

[139] UNEP/CBD/COP/DEC/XI/21 on Biodiversity and climate change: integrating biodiversity considerations into climate-change related activities, *CBD*, (Hyderabad, India, 8-19 October 2012).

IV. The Convention on the Conservation of European Wildlife and Natural Habitats

(1) General Information on the Convention

The Convention on the Conservation of European Wildlife and Natural Habitats is a legally-binding instrument which covers the natural heritage of Europe and some parts of Africa. The convention was signed in 1979 and entered into force on 1st June 1982. The main aims of the convention are

> to conserve wild flora and fauna and their natural habitats, especially those species and habitats whose conservation requires the co-operation of several States, and to promote such co-operation.[140]

The Bern Convention is one of the first which also includes the intrinsic value of species in their preamble and represents an important and new step in wildlife conservation.[141] By October 2014, there were 51 Contracting Parties including the European Union.[142] For its Contracting Parties, the Bern Convention is legally-binding and must be implemented by way of appropriate and necessary legislative and administrative measures.[143] This applies to all species listed in one of the first three appendices to the Bern Convention.[144] Chapter III of the convention text establishes different standards of protection depending upon the threat status of a species.[145] Article 6 requires the Parties to protect animal species listed in Appendix II by taking “appropriate and necessary legislative and administrative measures”. In order to strictly protect these species, this Article includes the prohibition of deliberate capture, taking or killing, or deliberate disturbing or damage of breeding and resting sides. Additionally, the possession and internal trade of species listed in Appendix II is prohibited. Article 7 specifies the measures of protection for species listed in Appendix III. Although, these measures are less strict than those in Article 6, it calls on the Contracting Parties to take appropriate measures to avoid exploitation of the animal species

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[140] Art. 1, *Bern Convention*.

[141] Preamble, *Bern Convention*; *Bowman/ Davies/ Redgewell*, Lyster`s International Wildlife Law (ed. 2; 2010), Cambridge University Press 297-344, 298.

[142] Art. 19(1), 20(1), *Bern Convention*.

[143] Art. 2-7, *Bern Convention*; *Bowman/ Davies/ Redgewell*, Lyster`s International Wildlife Law (ed. 2; 2010), Cambridge University Press 297-344, 333.

[144] Four Annexes are provided by the Bern Convention: Annex 1- strictly protected flora species, Annex II Strictly protected fauna species, Annex III- protected fauna species, Annex IV- Prohibited means and methods of killing, capture and other forms of exploitation.

[145] Art. 5-7, *Bern Convention*.

listed.[146] Furthermore, Article 12 gives Contracting Parties the opportunity for stricter measures to ensure the aim of this convention is reached.

The Secretary General of the Council of Europe presents the administrative part of the Bern Convention.[147] The decision-making body of the Bern Convention is presented by the Committee of Ministers. This Committee of Ministers has to approve the provisions and final recommendations before they are signed by the Contracting Parties.[148] Moreover, the Committee of Ministers can invite non-Contracting Parties to accede.[149] The monitor and reviewing body of the Bern Convention is represented by the Standing Committee with the opportunity for each Contracting Party to join.[150] Non-Contracting Parties, international or national as well as non-governmental bodies could be part of the Committee as observer.[151] The Group of Experts and the reporting system monitor the implementation of the recommendations of the Standing Committee.[152] Furthermore, the Standing Committee examines and adopts recommendations and resolutions on measures that need to be taken to improve the effectiveness of the convention. Recommendations and resolutions represent types of guidance documents to all Contracting Parties. They are not legally binding, however, of high relevance as documents setting standards. Recommendations are prepared by the Group of Experts. Currently, nine Groups of Experts with different scientific and conservation focuses exist.[153] They are composed of representatives of the Contracting Parties who are specialized in certain issues and can be supported by non-governmental organizations (NGOs).[154]

(2) Hybridization in the context of Bern Convention

In the context of the study at hand it is of particular interest whether a convention which focuses on the improvement of concrete conservation problems at particular sites[155] includes regulations on the treatment of hybridization and hybrids. At a first glance, no provisions on hybrids are provided in the convention text. Furthermore, it is not

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[146] Art. 7, *Bern Convention*: “measure to be taken shall include: (a) closed seasons and/or other procedures regulating the exploitation; (b) the temporary or local prohibition of exploitation, as appropriate, in order to restore satisfactory population levels; (c) the regulation as appropriate of sale, keeping for sale, transport for sale or offering for sale of live and dead wild animals”.

[147] *Ibid.*, Art. 16.

[148] *Ibid.*, Art. 16(2).

[149] *Ibid.*, Art. 14(1).

[150] *Ibid.*, Art. 13(1).

[151] *Ibid.*, Art. 13(2).

[152] *Ibid.*, Art. 14(2).

[153] *Council of Europe*, www.coe.int/t/dg4/cultureheritage/nature/experts_en.asp, last accessed March 2015.

[154] *Ibid.*

[155] *Bowman/ Davies/ Redgewell*, Lyster`s International Wildlife Law (ed. 2; 2010), Cambridge University Press 297-344, 338.

immediately clear whether hybrids would obtain the same legal status as their pure relatives listed in one of the appendices considering that a definition of the genetic status of a species as well as a definition of a hybrid is not included in the convention text.[156] The term 'hybrid' appeared for the first time in a recommendation on the eradication of the ruddy duck in 2010, recognizing the threat of hybridization with this non-native species for the native white-headed duck.[157] In its recommendation the Standing Committee explicitly requests the total eradication of the ruddy duck including the hybrids of both species. In order to ensure the aim of total eradication, the action plan (II) proposed furthermore to "phase out all captive populations of Ruddy Ducks, if possible by 2020", to avoid new escapes to the wild. However, the document does not contain either a definition of the term 'hybridization' or 'hybrid' or a statement concerning the definite identification of hybrids.

Hybridization between wolves and domestic dogs is a common phenomenon in Europe and could weaken the genetic integrity of native wolf populations as well as their behavior.[158] In 2000, the possible threat of domestic species for their wild relatives was recognized by the Group of Experts on Conservation of Large Carnivores to the Bern Convention in the Action Plan for Wolves.[159] Although the authors support the removal of feral and stray dogs, they argue that it must be regulated and conducted by authorized persons. Otherwise, illegal killing of wolves is to be feared as well as conflicts with animal rights groups.[160] In order to achieve a better understanding of the distribution and degree of hybridization, the experts recommend actions to improve the genetic identification of hybrids.[161] This Action Plan represents the first, although not legally binding, species-specific approach concerning a hybrid of a domesticated animal and its wild congener in a document of an international environmental treaty. In co-operation with the Large Carnivore Initiative of Europe (LCIE)[162] the 'Carnivore Guidelines' were developed and

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[156] *Trouwborst*, RECIEL 23.1 (2014): 111-124, 115.

[157] Council of Europe, *Standing Committee*, Recommendation No. 149 (2010) of the Standing Committee on the eradication of the Ruddy Duck (*Oxyura jamaicensis*) in the Western Palaearctic;

[158] *Randi*, Detecting hybridization between wild species and their domesticated relatives, *Molecular Ecology* (2008), 285-293, 285; Council of Europe, *Standing Committee*, prepared by *A. Trouwborst,* Applying the Bern Convention on the Conservation of European and Natural Habitats to the Problem of Hybridization between wolves (*Canis Lupus*) and domestic dogs – A Analysis and a Proposal for a Standing Committee Recommendation, (T-PVS/Inf (2014) 15), 7.

[159] *Ibid*., 8. Council of Europe, *Group of Experts,* established by *Boitani,* (2000) on Conservation of Large Carnivores – Action Plan for the conservation of the wolves (*Canis lupus*) in Europe, (T-PVS (2000) 23), 4.7.5., 34.

[160] *Ibid*., 35.

[161] *Ibid*., 41 (4.12.1.).

[162] Specialist group within the Species Survival Commission (SSC) of the IUCN.

endorsed by a recommendation in 2008.[163] The authors agreed on the recommendation that the removal of hybrids from nature by lethal control or live capture is necessary to protect wolf populations, however, they also point out the problem of the missing conservation status of wild-born (wolf-dog) hybrids.[164] They propose:

> […] Their management status should be such that they receive the same legal status as wolves from hunters and the public in order to close a potential loophole for the irregular killing of wolves - but such that they can be effectively removed under special license by carefully trained government appointed wardens when necessary. […].

The LCIE also underlines the lack of knowledge in hybrid identification and management[165] and requests an increased research. In 2010, the LIFE+ Nature & Biodiversity program decided on funding the project “IBRIWOLF - Pilot actions for the reduction of the loss of genetic patrimony of the wolf in central Italy”.[166] LIFE (L'Instrument Financier pour l'Environnement) was developed as a funding instrument to support environment and climate actions in order to improve EU environmental and climate policy and legislation.[167] The project `IBRIWOLF´ started in Italy in 2011 and represents a pilot study on the management of anthropogenic hybridization and hybrids. One major aim was to prepare guidelines for the management of wolf-dog hybrids and to improve the identification of hybrids. In 2012, the Standing Committee to the Bern Convention also invited Italy to develop a strategy to deal with the hybridization problem of wolves and dogs in a recommendation on “large carnivore populations in Europe requesting special conservation action".[168] This project already enabled an improvement of methods for the identification of wolf-dog hybrids. Furthermore, public relation activities supported the awareness to this problem and management guidelines were developed in order to support environmental policy.[169] Effectively, this project gave assistance for an

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[163] Council of Europe, *Standing Committee*, Recommendation No. 137 (2008) on Population Level Management of Large Carnivore Populations, (T-PVS/Inf(2008)17E); *European Commission*, Note to the Guidelines for Population Level Management Plans for Large Carnivores, 1 July 2008.

[164] *See also Trouwborst*, RECIEL 23.1 (2014): 111-124, 123.

[165] *Linell/ Salvatori/ Boitani*, (2008): 77- 78.

[166] LIFE10 NAT/IT/00026; The LIFE programme is managed by the *European Commission* and promotes the “implementation, updating and development of European environmental policy legislation by co-financing projects with European added value”.
See also: http://ec.europa.eu/environment/life/about/index.htm, last accessed November 2014.

[167] http://ec.europa.eu/environment/life/about/index.htm, last accessed November 2014.

[168] Council of Europe, *Standing Committee* (T-PVS/Inf (2014) 15, 8); Council of Europe, *Standing Committee*, Recommendation No. 162 (2012) on the conservation of large carnivores populations in Europe requesting special conservation action.

[169] http://www.ibriwolf.it/en/content/results, last accessed December 2014.

"Analysis and Proposal for a Standing Committee Recommendation" concerning the problem of wolf-dog hybrids in 2014.[170] For purposes of the implementation of this draft recommendation, the author Trouwborst defined the term 'wolf-dog hybrid' "as meaning a wild living animal with both wolf and dog ancestry which can be confirmed by the current taxonomic techniques (using both morphological and genetic features)".[171] This constitutes the first draft of a definition of a hybrid within the Bern Convention. However, it is species-specific and not legally binding. In December 2014, the Standing Committee took note of this proposal in a corresponding recommendation[172] and noted, *inter alia*,

> the need to address these challenges through effective preventive and mitigation measures, including the detection of free-ranging wolf-dog **hybrids** and their government-controlled removal from wild wolf populations exclusively by bodies entrusted with this responsibility by the competent authorities.

Furthermore, it recommends the Contracting Parties to:

> 1. Take adequate measures to monitor, prevent and mitigate **hybridisation** between wild wolves and dogs, including, as appropriate, effective measures to minimise numbers of feral and stray (free-ranging) dogs, and the prohibition or restriction of the keeping of wolves and wolf-dog hybrids as pets;
> 2. Take action to promote the detection of free-ranging wolf-dog **hybrids**, and to ensure government-controlled removal of detected wolf-dog hybrids from wild wolf populations;
> 3. Ensure that the government-controlled removal of wolf-dog **hybrids** takes place after government officials and/or the bodies entrusted by government for this purpose and/or researchers have confirmed them as hybrids using genetic and/or morphological features. Removal should only be carried out by bodies entrusted by the competent authorities with such a responsibility, while ensuring that such removal does not undermine the conservation status of wolves;
> 4. Adopt the necessary measures to prevent wolves from being intentionally or mistakenly killed as wolf-dog **hybrids**. This is without prejudice to the careful government-controlled

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[170] Council of Europe, *Standing Committee*, prepared by *A. Trouwborst*, Applying the Bern Convention on the Conservation of European and Natural Habitats to the Problem of Hybridization between wolves (*Canis Lupus*) and domestic dogs – A Analysis and a Proposal for a Standing Committee Recommendation, (T-PVS/Inf (2014) 15).

[171] *Ibid*. 18.

[172] Council of Europe, *Standing Committee*, Recommendation No. 173 (2014) on Hybridisation between wild gray wolves (Canis lupus) and domestic dogs (Canis lupus familiaris).

> removal of detected wolf-dog hybrids from the wild by bodies entrusted with this responsibility by the competent authorities.

Fourteen years after the problem of wolf-dog hybrids was recognized by the Group of Experts noted in the Action Plan for Wolves, the first recommendation was developed by the Bern Convention. Although a definition of the term ‘hybrid’ was presented in the proposal for this recommendation, it is missing in the final recommendation. Nonetheless, the Standing Committee recommends the removal of hybrids by experts only if the status is proven in order to prevent illegal killings. Furthermore, a prohibition to keep wolves and wolf-dog hybrids as pets is suggested to reduce the probability to hybridize with their wild congener.

The aforementioned examples underline the complexity of the topic hybridization which certainly contributed to the existing legislative difficulties and uncertainties. Thus, in spite or even because of the continuing uncertainties in cases of hybrids and hybridization the application of the precautionary approach, as applied in the aforementioned recommendations, is justified and may represent an appropriate measure to implement even legally binding provisions. Nonetheless, an increased research and an improvement of methods such as genetic techniques is suggested in order to built the necessary basis for the development of legal instruments dealing with hybrids. Moreover, both examples represent non-legally binding species-specific approaches and it therefore remains doubtful, considering the idiosyncratic character of hybridization, if such guidelines could be transferred to hybridization processes of other species or hybridization processes of another anthropogenic origin.

V. Preliminary Conclusion

In summary, it can be concluded that the three most important international environmental treaties, even though no reference is made to it in any of the convention texts, recognized the problem of hybridization as threat to biodiversity, particularly as a consequence of the introduction of non-native species and domesticated species. Despite the apparent efforts of decision-makers and experts of all three conventions to tackle this problem (e.g. guidelines and management tools), there are neither legally binding definitions of the terms ‘hybridization’ and ‘hybrid’ nor concrete or legally binding provisions on how to deal with hybridization and its consequences for species conservation. For States parties to CITES only a non-legally binding resolution (Res. Conf. 10.17) exists, which deals with hybrids emerging from the interbreeding of species where at least one is included in the appendices to CITES. Such a resolution represents a useful framework for the appropriate implementation of CITES into national law by the States parties. However, it must be considered that this resolution only concerns the trade of hybrids to avoid overexploitation of the purebred species in the wild, whereas the legal status of hybrids in the wild and their role as a threat to biodiversity is not considered. The CBD is more reserved concerning the question how to deal with this problem and asks for increased research. Nonetheless, the CBD recognized hybridization as a potential threat in three thematic areas (invasive alien species, island biodiversity, climate change) which underlines the complexity of this topic. Besides the general resolution on hybrids presented by CITES, the Bern Convention presented two species-specific recommendations dealing with hybrids. While CITES aims at a conservation status for hybrids (i.e. treats them similar to the threatened parental species), the Bern Convention proposes both a conservation status as well as the eradication or removal of non-native hybrids. However, the purpose of the aforementioned conservation status is to avoid irregular killings of the native species, not to protect hybrids. If a conservation of hybrids is more effective in order to preserve biodiversity or if a species-specific approach including the eradication of hybrids is useful, is discussed in more detail in the final conclusion. However, this suggests that a legally binding definition of hybridization and hybrids should be elaborated by all three conventions in a harmonized way. Scientific uncertainty and the complexity of this topic are assumed to be the main reasons for the lack of a legally binding provision. Nonetheless, all three conventions refer to the precautionary approach, however, in non-legally binding documents (i.e. preamble, resolution or recommendations), which enables the respective Parties to adopt and implement legally binding provisions even in the case of scientific uncertainty, in particular where there is conclusive evidence for a significant threat to biodiversity. Thus, the application of the precautionary approach in the case of hybridization or hybrids and therefore, the development of legally binding provisions are clearly justified. However, the existing approaches towards hybridization suggest that currently the precautionary approach plays a more important role in helping to guide and develop hybridization related measures rather than to implement legally binding provisions.

C. European environmental law in the context of hybridization

I. General Information on the EU and the EU environmental policy

In order to investigate the role of hybridization in European law, a detailed consideration of the European environmental policy is necessary. The European Union (EU) is a unique supranational organization that has currently 28 Member States.[173] The competences to prescribe are distributed between the EU institutions and the Member States in accordance with the principle of conferral.[174] European law is based on the rule of law and enjoys supremacy vis-à-vis national law.[175] These particular legal effects make it necessary to distinguish it from 'traditional' international law. In Article 191 of the Treaty on Functionality of the European Union (TFEU title XX)[176] the objectives of the European environmental policy are determined as follows:

> […] preserving, protecting and improving the quality of the environment, protecting human health, prudent and rational utilization of natural resources, promoting measures at international level to deal with regional or worldwide environmental problems, and in particular combating climate change. […].

Article 192 (1) TFEU then serves as the jurisdictional basis for enacting environmental measures, in particular by way of adoption of regulations and directives.

Due to the varied scope and issue of hybrids and hybridization the European Union environmental policies, whose specific context is the precautionary principle, is subject for discussion.[177] However, no definition of what is meant by this principle exactly is mentioned in the TFEU. In 2000, the European Commission presented a non-binding Communication on the precautionary principle to form an understanding of this principle.[178] The application of this principle is justified, if the "identification of potentially negative effects resulting from a phenomenon, product or process;" is possible, even though "a scientific evaluation of the risk which because of the insufficiency of the data, their inconclusive or imprecise nature, makes it impossible to determine with sufficient

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[173] Treaty on the Functioning of the European Union (TFEU) consolidated version 2012 (OJ C 326, 26 October 2012). *See also* http://europa.eu/about-eu/index_en.htm, last accessed August 2014.

[174] Art. 5, *TFEU.*

[175] *Epiney*, Umweltrecht in der Europäischen Union, Heymanns (2005), 114.

[176] Treaty on the Functioning of the European Union (TFEU) consolidated version 2012 (OJ C 326, 26 October 2012).

[177] Art. 191 (2), *TFEU XX.*

[178] *European Commission*, COM (2000) 1 final, Communication from the European Commission on the precautionary principle.

certainty the risk in question". With regard to the topic at hand, anthropogenic hybridization is known as a phenomenon which can threaten a species with extinction, whereby the causes and consequences remain often unknown. These scientific uncertainties call for an application of the precautionary principle if hybridization is recognized as a potential threat to biodiversity.

In the following section, the application of CITES provisions by the European Union will be elaborated taking into account that the European Union is one of the "world´s biggest trader"[179] and CITES is the only international organization with a specific resolution on hybrids.[180] Furthermore, this section aims to point out the role of the Habitats Directive[181] as one of the most important European legal instruments required by the CBD, to which the European Union is party since 1993.[182] Additionally, the possible role of other European environmental policies is examined.[183]

II. The Implementation of CITES by the EU

The global trade of wild flora and fauna is an important economic sector and of enormous importance for the EU. In the following analysis the application of CITES by the EU is analyzed, especially with regard to the only international resolution on hybrids.

It was only in 2013 that the ability to accede to CITES as a "regional economic integration organization constituted by sovereign states" was given to the EU.[184] In September 2013, a sufficient number of Parties had ratified the "Gaborone amendment"[185] to Article XXI of the convention which entered into force in accordance to Article XVII 3 in November 2013. The amendment contains, *inter alia,* two new paragraphs:

> […] 2. This Convention shall be open for accession by regional economic integration organizations constituted by sovereign States which have competence in respect of the

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[179] *European Commission* (2014) European Union Trade and Investment 2014, 3. doi:10.2781/65243.
[180] *See* B. II.
[181] *Council of the European Communities*, Habitats Directive, Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora (21 May 1992).
[182] Art. 6, *CBD.*
[183] The analysis of the Bern Convention has shown that the problem of wolf-dog hybrids is also taken into account by the EU which is also a Member State of the Bern Convention. In order to prevent overlaps, this topic is only discussed in section B) IV) regarding that even though the LCIE is strictly speaking not an integral part of the EU environmental policy, it addresses potential loopholes of irregular killing as well as supporting the development of a conservation status of wild-born hybrids including the protection of those within the European legislation.
[184] *CITES* Notification to the Parties No. 2013/045 (Geneva, 4 October 2013), entry into force of the amendment to Article XXI of the text of the Convention (Gaborone, 30 April 1983). *See also* http://cites.org/eng/news/pr/2013/20131003_gaborone.php, last accessed December 2014.
[185] *Ibid.*

> negotiation, conclusion and implementation of international agreements in matters transferred to them by their Member States and covered by this Convention. […].
>
> 4. In matters within their competence, such regional economic integration organizations shall exercise the rights and fulfill the obligations which this Convention attributes to their Member States, which are Parties to the Convention. In such cases the Member States of the organizations shall not be entitled to exercise such rights individually. […].

In the perspective of accession to CITES, the EU would need to implement this convention including the respective regulations into European law. In return, the "Gaborone amendment" would give the EU a voice in work of CITES and would enable access to the financial support given by it for States parties.[186] Furthermore, three main reasons are pointed out by CITES World Article (section "The European Union")[187] for an implementation of CITES on EU level rather than individually by the EU Member States:

- the fact that external trade rules are of exclusive Community competence;
- the absence of systematic border controls as a result of the customs union; and
- the existence of a Community policy on the environment and legislation on the protection and conservation of the Community's indigenous species.

In December 2013, a corresponding proposal was published by the European Commission which is currently discussed by the Council of Europe and the European Parliament.[188] Nevertheless, the EU had implemented the provisions of CITES already before this amendment entered into force due to the fact that nearly all Member States are also States parties to this convention.[189] CITES is, therefore, implemented by the EU and is regulated by the Council Regulation (EC) No. 338/97[190] and the Commission Regulation (EC) No. 865/2006[191] which are amended with the Commission Regulation (EU) No. 750/2013[192],

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[186] *European Commission* (2012), Convention on international trade in endangered species of wild flora and fauna (CITES) - Gaborone Amendment to the Convention- Information pack for Parties, 3-4.

[187] *CITES* World, Official Newsletter of the Parties Issue Number 15 (July 2005), 14.

[188] *European Commission,* COM (2013) 867 final, Proposal for a Council Decision on the Accession of the European Union to the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), Brussels, 6th December 2013.

[189] *Ibid.*, 4.

[190] *European Commission* , Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein (OJ 1997 L 61, 1).

[191] *European Commission*, Commission Regulation (EC) No 865/2006 of 4 May 2006 laying down detailed rules concerning the implementation of Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora by regulating trade therein (OJ 2006 L 166, 1).

[192] *European Commission*, Commission Regulation (EU) No. 750/2013 of 29 July 2013 amending Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora by regulating trade therein. (OJ 2013 L212/1).

No. 100/2008[193] and No. 791/2012.[194] The main differences between CITES and the EU Wildlife Trade Regulations are mentioned as follows:

- The EU regulations establish stricter import conditions than those imposed by CITES. Import permits are not only required for species listed in Annex A but also for species listed in Annex B. Import notifications are required for Annexes C and D.
- Some species that are listed in Appendix II of CITES are listed in Annex A of the EU regulations and consequently cannot be traded or used for commercial purposes.
- Live specimens of species listed in Annex A and B are only allowed to be imported into the EU if the recipient is suitably equipped to house and care for the specimens; CITES requires suitable care and housing only for imports of live Appendix I specimens.
- The EU regulations regulate trade within and between EU Member States - considered domestic trade - as well as international trade with non-EU Member States; CITES regulates international trade only.
- Regulation (EC) 338/97 authorises the EU Member States to suspend imports with regard to certain species and countries (negative opinions of the EU Scientific Review Group and EU import suspensions), even if trade is allowed under CITES.[195]

Council Regulation (EC) No. 338/97 disregarded wild-born animal hybrids in control of trade as well as the Commission Regulation (EC) No. 865/2006.[196] However, on the eve of the accession to CITES, the resolution on hybrids was more or less adopted by the Commission Regulation 750/2013, stipulated that:

> […] **Hybrids** may be specifically included in the Appendices but only if they form distinct and stable populations in the wild. Hybrid animals that have in their previous **four generations** of the lineage one or more specimens of species included in Annexes A or B shall be subject to the provisions of this Regulation just as if they were full species, even if

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[193] *European Commission*, Commission Regulation (EC) No 100/2008 of 4 February 2008 amending, as regards sample collections and certain formalities relating to the trade in species of wild fauna and flora, Regulation (EC) No 865/2006 laying down detailed rules for the implementation of Council Regulation (EC) No 338/97 (OJ 2008 L31/3).

[194] *European Commission*, Commission Regulation (EC) No. 791/2012 of 23 August 2012 amending, as regards certain provisions relating to the trade in species of wild fauna and flora, Regulation (EC) No 865/2006 laying down detailed rules for the implementation of Council Regulation (EC) No 338/97 (OJ 2012 L242/1).

[195] *European Commission*, Wildlife Trade Regulations in the European Union - An Introduction to CITES and its Implementation in the European Union (2010), 13, (doi: 10.2779/21758).

[196] Nevertheless, the term 'hybrid' occurs in this regulation. Article 62 (1) in accordance with Article 8 (*European Commission* , Council Regulation (EC) No 338/97) enables the trade of species listed in one of the annexes including their hybrids if they were captive bred. Due to the fact that captive bred hybrids were out of the scope of this study it is not discussed in more detail. An official definition for 'hybrid' is missing.

> the hybrid concerned is not specifically included in the Annexes. […].[197]

In pursuance of CITES these regulations only concern the trade of wild-born hybrids and not the legal status of hybrids in the wild or their effects on biodiversity conservation. Nevertheless, including hybrids in the European Wildlife Trade Regulation will improve the conservation of threatened species by impeding the circumvention of laws by trading entities tagging pure species as hybrids. Only the trade of demonstrably captive-bred hybrids should be possible under these regulations. This regulation is a well justified application of the precautionary principle. Considering the uncertainty of distinguishing hybrids and their purebred relatives, such an obligation represents an adequate measure in order to prevent loopholes for trading entities and therefore, to fulfil the objectives of this regulation.

III. Hybridization and hybrids under the Habitats Directive

The EU has acceded to the CBD. In order to implement the obligations contained therein, the EU has developed a European biodiversity strategy.[198] The Habitats Directive is a central part of this strategy and thus plays an important role in respect of biodiversity conservation within Europe. Especially with a view to the hybridization and hybrids issues, this piece of legislation will be further analyzed. Originally, the Habitats Directive must be seen as a legal mechanism to implement the Bern Convention.[199] Based on the model of the Bern Convention the aim of this Directive is to ensure

> […] biodiversity through the conservation of natural habitats and of **wild** fauna and flora in the European territory of the Member States to which the Treaty applies. […].[200]

Article 12 of the Habitats Directive asks for the “strict protection” of animal species by implementing appropriate measures.[201] Article 12 of the Habitats Directive and Article 6 of

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[197] *European Commission*, Commission Regulation (EU) No. 750/2013 of 29 July 2013 amending Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora by regulating trade therein. (OJ 2013 L212/3), entered into force on the 10.08.2013 (Article 2).

[198] *European Parliament*, Resolution of 20 April 2012 on our life insurance, our natural capital: an EU biodiversity strategy to 2020 (2011/2307(INI)).

[199] Convention on the Conservation of European Wildlife and Natural Habitats (19 September 1979); in force 1 November 1983 (Bern Convention). *The Council of the European Communities*, Council Directive 92/43/EEC (21 May 1992) on the Conservation of Natural Habitats and of Wild Fauna and Flora (Habitats Directive), (21 May 1992), (OJ 1992 L206).

[200] Art. 2 (1), *Habitats Directive,* (OJ 1992 L206/9).

[201] *Ibid.*, Art. 12 *Habitats Directive* (OJ 1992 L206/12).

the Bern Convention show similar aims but due to differences in Annex IV (a) (Habitats Directive) and Annex II (Bern Convention), the European Court of Justice (CJEU)[202] ruled that the Habitats Directive did not entirely coincide with the Bern Convention.[203] Thus, the autonomous character of the obligations under the Habitats Directive is maintained and the obligations of both, Habitats Directive and Bern Convention, apply to their Contracting Parties.[204]

Two Annexes (IV, V)[205] are provided by the Habitats Directive for animal and plant species protection. Therefore, Articles 12-15 of the Directive specify different protection measures depending on the annex they are listed on which should ensure an efficient conservation. Taking into account that more than one threat to an animal species could exist, a species can be listed in more than one annex. In this case, different conservation measures may be applicable to a particular species.[206] For this reason, the Habitats Directive gives Member States the opportunity for a flexible implementation of the legally binding Directive by a “species-by-species” approach.[207] With regard to hybridization, such a “species-by species” approach appears to be an adequate instrument for the implementation of specific management plans for the prevention of hybridization processes or dealing with hybrids. Nonetheless, neither hybrids nor hybridization are directly mentioned by the Habitats Directive or the European Commission’s general guidance document on the strict protection of animals.[208] Article 12 of the Habitats Directive requests the Member States to implement measures to prohibit “all forms of deliberate capture or killing of specimens in the wild;” of animal species listed in Annex IV. Similar to the Bern Convention, even the Habitats Directive does not define the term “wild” as 100% genetically pure species. Thus, a specimen with a mixed genome could either fall in the scope of the Habitats Directive or not, depending on interpretation.[209] However, which

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[202] Article 13, 19, *TEU*.

[203] *European Court of Justice* (CJEU), Case C-75/01, Commission v. Luxembourg (2003); I- 1644, recital 55-57; *European Commission*, Guidance document on the strict protection of animal species of Community interest under the Habitats Directive 92/43/EEC, 8, “Two important differences should be stressed. Firstly, the Convention has a greater species coverage, since its Appendix II contains a larger number of species than Annex IV(a) of the Directive (partly due to the larger geographic area covered by the Convention). Secondly, the word “deliberate” figures in Article 6(b) but is absent from Article 12(1) (d)”.

[204] *European Court of Justice* (CJEU), Case C-75/01, Commission v. Luxembourg (2003); I- 1644, recital 55-57; *European Commission*, Guidance document on the strict protection of animal species of Community interest under the Habitats Directive 92/43/EEC, 8.

[205] *The Council of the European Communities*, Council Directive 92/43/EEC, 50, 62), “Annex IV: Animal and plant species of community interest in need of strict protection. Annex V: Animal and plant species of community interest whose taking in the wild and exploitation may be subject to management measures”.

[206] *Ibid.*, 14 (34), *European Commission*.

[207] *Ibid.*, 15 (36); 20 (57).

[208] *Trouwborst*, RECIEL 23.1 (2014): 111-124, 112.

[209] *Ibid.*, 115.

interpretation is most useful in terms of protection depends on the impact of hybridization on the species involved and the causes for hybridization. From this, it can be concluded that only if anthropogenic hybridization leads to hybrid offspring which negatively affects the population of the native species, a removal of this hybrid is recommended. Therefore, in this case it would not be appropriate that hybrids obtain the same legal status under the Habitats Directive as their pure parental species. Nevertheless, a removal or killing should be regulated and only permitted if any mistaking with wild native relatives can be excluded. Furthermore, such a removal should be target-aimed and cost-effective. If hybridization of wild species emerges from unstoppable anthropogenic environmental changes, hybridization may also be an advantage for the species involved as it may facilitate a better adaptation to new environmental conditions. In this case, a removal of hybrids would be counterproductive.

Interestingly, although hybrids are not explicitly mentioned in the regulatory text of the Habitats Directive, the hybrid taxon *Pelophylax esculentus* (formerly known as *Rana esculenta*)[210] is listed in Annex V to the Habitats Directive. Unlike other hybrids where gametes contain mixed parental genomes, *Pelophylax esculentus* show no complete admixture. By contrast, half of each parental genome is transmitted intact from generation to generation (hybridogenesis or hemiclonal mode of reproduction). This hybridogenesis enables *Pelophylax esculentus* to backcross with both parental species as well as to form stable hybrid populations under certain circumstances. [211] This unusual way of hybridization renders it difficult to categorize this taxon in accordance with the Biological Species Concept.[212] However, the denomination of this taxon with a species name as well as its inclusion in Annex V to the Habitats Directive ignores this scientific uncertainty and thus does not restrain decision-makers from declaring an independent conservation status

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[210] *Pelophylax esculentus* (before *Rana esculenta*) is the fertile hybrid of two frog species *Pelophylax ridibundus* (before *Rana ridibunda*) and *Pelophylax lessonae* (before *Rana lessonae*).

[211] Only *Pelophylax esculentus* hybrids with two separated genomes emerge from hybridization. Backcrosses with one parental species may lead to diploid or triploid hybrid offspring (a half of the parent genome is transmitted to the hybrid genome) or to the emergence of a pure parental species (parent genome combined with the respective half hybrid genome). *Beebee*, Ecology and Conservation of Amphibians, Conservation Biology Series, Chapman and hall, London (1996), 57-58. *Heppich,* Hybridogenesis in Rana esculenta: C- band karyotypes of Rana ridibunda, Rana lessonae and Rana esculenta, *Journal of Zoological Systematics and Evolutionary Research* 16.1 (1978): 27-39, 27.

[212] *Duboi/ Günther,* Klepton and synklepton: two new evolutionary systematics categories in zoology. Zool. Jahrb. Syst.(1982) 109: 290-305. Considering that clonal hybrids represent neither a real species nor a typical hybrid in the sense of the BSC, the authors proposed a new evolutionary systematic category (i.e. “klepton”) in order to facilitate the assignment of a taxon. Therefore, the abbreviation “kl.” is used in between the binary nomenclature in order to denote a “klepton” species. By contrast, other authors prefer, indeed, a formally naming of hybrids as they are more stable in time than others., however, strictly reject a new species category as mentioned by *Dubois and Günther*. *Hotz et al*. Are hybrid clonals species? A case for enlightened anarchy, Amphibia-Reptilia (1996) 17.4, 315-320, 317-318.

of this hybrid taxon.

Currently, *Pelophylax esculentus* is classified on the IUCN Red List of Threatened Species under the category[213] "Least Concern", albeit with a decreasing population trend.[214] Due to its special biology and its ability to form permanent populations, an independent conservation status of this species is legitimate. This special case of hybridization also shows that the scientific decision of declaring a taxon a species can have considerable legal consequences. The preservation of this hybrid taxon is justified as it is unlikely to threaten the parental species, because the genomes are not admixed. It may rather represent a "vehicle" for these genomes which may facilitate dispersal and persistence of the genetic integrity of the parental species.

In order to avoid hybridization of sympatric species, the protection of natural habitats and thus the existing ecological barriers, independent of the legal status of a hybrid, is important and presents a precautionary approach for the conservation of biodiversity.[215] The Habitats Directive is a suitable instrument for the conservation of biodiversity that may be further improved by appropriate legislative amendments or guidelines for implementation regarding hybridization and hybrids.

IV. The role of hybridization in approaches towards a legislation of invasive alien species in the EU

Invasive alien species represent one of the major threats to biodiversity loss with enormous ecological impacts.[216] The "Technical Support to EU Strategy on Invasive Alien Species (IAS)", published in 2009, mentioned hybridization as one of the main ecological effects of IAS.[217] In 2013, the European Commission submitted a "proposal for a Regulation of European Parliament and the Council of Europe on the prevention and management of the introduction and spread of invasive species"[218]. The following year on 4 November 2014,

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[213] *IUCN, The IUCN Red List of Threatened Species. Version 2014.3*. http://www.iucnredlist.org, last accessed March 2015. The aim of the Red List is: "To provide information and analyses on the status, trends and threats to species in order to inform and catalyse action for biodiversity conservation*.*" Seven categories are provided by the IUCN: least concern, near threatened, vulnerable, endangered, critically endangered, extinct in the wild and extinct.

[214] *Kuzmin et al. Pelophylax esculentus*. The IUCN Red List of Threatened Species (2009). Version 2014.3. www.iucnredlist.org, last accessed March 2015.

[215] *Honeycutt et al.*, Molecular Approaches in Natural Resource Conservation and Management, Cambridge University Press (2010): 1-35, 3.

[216] *Kettunen et al.*, Technical support to EU strategy on invasive alien species (IAS) Assessment of the 353 impacts of IAS in Europe and the EU. Brussels: Institute for European Environmental Policy (2009)*, 1, 5.*

[217] *Ibid*., 5, 8, 12.

[218] *European Commission*, COM (2013) 620 final, Proposal for a Regulation on the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species, 7 (3).

the European Parliament and the Council of the European Union have taken up the suggestions of the European Commission and passed a regulation on the prevention and management of the introduction and spread of invasive alien species[219] which also concerns the threat of hybridization:

> […] The threat to biodiversity and related ecosystem services that invasive alien species pose takes different, including severe impacts on native species and the structure and function of ecosystems through the alteration of habitats, predation, competition, the transmission of diseases, the replacement of native species throughout a significant proportion of range and through genetic effects by **hybridisation**. […].[220]

Furthermore, in Article 3 (1) a definition of 'alien species' is given which includes also hybrids of alien species and native species[221]:

> (1) 'alien species' means any live specimens of species, subspecies or lower taxon of animals, plants, fungi or micro-organisms introduced outside its natural range; it includes any part, gametes, seeds, eggs, or propagules of such species, as well as any **hybrids**, varieties or breeds that might survive and subsequently reproduce;

Apart from this definition, this regulation specifies the term "invasive alien species" as follow[222]:

> (2) 'invasive alien species' means an alien species whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services;

Nonetheless, a precise definition up to which generation a hybrid would fall within the scope of the aforementioned article is still missing. On the one hand, it could be interpreted as the first generation (F1) of a crossbreed between an invasive species and a native species. On the other hand, it could also include individuals that show any degree of introgression by an invasive alien species. However, this is the first time that the term

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[219] *European Parliament and the Council of the European Union,* Regulation (EU) No. 1143/2014 of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. (OJ 2014 L317/35-55), entered into force on the 01.01.2015, (Art. 33).
[220] *Ibid.,* OJ 2014 L317/35.
[221] *Ibid.,* OJ 2014 L317/44, Art. 3 (1).
[222] *Ibid.,* OJ 2014 L317/44, Art. 3 (2).

‘hybrid’, even in the absence of a separate definition is included in a legally binding document.

Chapter II of this regulation deals with the prevention of invasive alien species and includes six articles. Although hybridization is not specifically mentioned in these provisions, prevention of invasive alien species indirectly also means avoidance of hybridization. Article 17 of this regulation requests the “rapid eradication at an early stage of invasion”.[223] This implies that also hybrids of invasive alien species have to be eradicated considering this obligation. It suggests that in order to protect native species against introgression of the genome and genetic displacement by invasive alien species, the eradication of hybrids and their offspring is an adequate opportunity to minimize this risk. Nonetheless, a conflict of laws and policies may arise, if hybrids of an invasive alien or domesticated species and a native species listed in the annexes of the Habitats Directive or other relevant annexes emerge.[224] Although this regulation on IAS is ambitious, an improvement of the efficiency with regard to hybrids is possible. In addition to updates and improvements proposed by other authors[225], a precise definition of hybrids including a concrete statement concerning the question up to which generation an individual would fall under this category would improve the strategy how to deal with IAS.

V. Preliminary Conclusion

The comparison of the different approaches of the European Wildlife Trade Regulation, the Habitats Directive, the regulation on IAS raises questions on the conservation status of wild-born hybrids. If hybrids of an invasive alien or domesticated species and a native species listed in the annexes of the Habitats Directive or other relevant annexes emerge, a conflict of laws and policies may arise. While the abovementioned regulation on IAS and the proposals of the LCIE would favor the rapid eradication of these hybrids, they would obtain a conservation status under the European Wildlife Trade Regulation and, depending on the interpretation, also under the Habitats Directive. Considering the provision of the European Wildlife Trade Regulation, a trade and, depending on the interpretation, also a removal is not permitted until the fifth generation of a hybrid. Nonetheless, an eradication

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[223] Ibid., OJ 2014 L317/49, Art. 17 (1), “After early detection and within three months after the transmission of the early detection notification referred to in Article 16, Member States shall apply eradication measures and notify those measures to the Commission and inform the other Member States”.

[224] *See* B. II.

[225] *Beninde/ Fischer /Hochkirch/ Zink*, Ambitious advances of the European Union in the legislation of invasive alien species, Conservation Letters (2014) DOI: 10.1111/conl.12150, 13. Such as: Shorter monitoring intervals, elucidating valuation of species and improvement of funding.

or removal of hybrids from the wild would be useful to protect native wild species against introgression and negative effects of hybridization. This analysis reveals that with respect to consequences of hybridization, the approaches concerning the conservation status of hybrids in the European environmental policy are diverse and complex. The complexity and discordance between the single instruments represent significant legal uncertainties which may explain the lack of an official legal definition or legal instruments dealing with hybrids.

The only legally binding regulation on hybrids (with comments up to which generation it applies) within the European environmental policy is the European Wildlife Trade Regulation, in which hybrids until their fourth generation should receive the same legal status as their parental species, if at least one of the parental species is listed in the annexes. The aforementioned regulation was reached in face of the potential loophole for trading entities to declare purebred species as hybrids to circumvent trade restrictions.[226] This regulation only concerns the trade of species and has no direct relevance for the management of hybrids in the wild. The regulation "on the prevention and management of the introduction and spread of invasive alien species" demands, on the contrary, the eradication of invasive alien species including their hybrids. However, the possibility that hybrids could concurrently fall within the scope of more than one provision is disregarded by all approaches of the EU environmental policy and may cause legal uncertainty.

Climate Change and habitat destruction as facilitators of hybridization are not considered by any European environmental policy. Nonetheless, the protection of natural habitats under the Habitats Directive could also be interpreted as a measure in order to prevent hybridization by maintaining ecological barriers between sympatric species.

In conclusion, it can be stated that the European environmental policy is currently imprecise concerning the problem of hybridization and hybrids, with only a few approaches, which were usually added later as a response of an emerging problem. Interestingly, although the specific context of the European environmental policy is the precautionary principle, concrete and precise recommendations how to derive management plans or conservation strategies considering the threat of hybridizations as well as concrete legally binding definitions are missing. This may be attributable to the insufficient scientific knowledge or to an underestimation of the associated risk of hybridization which restrains decision-makers from making concrete recommendations. However, the situation is rather similar to the situation in public international law.

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[226] *See* B. II.

D. National environmental law in the context of hybridization

Although the threat of hybridization and hybrids is a global topic and has to be regulated in a transboundary context, the approach of national governments in terms of hybrids and hybridization is important to ensure the implementation and success of international and European environmental policies. Despite existing provisions of international and European environmental law, the implementation varies among the Member States.[227] Furthermore, States outside of international organizations and European law may pursue additional strategies in nature conservation. This in turn could provide a statutory basis for a further development in international environmental law and could provide useful examples for international provisions. In this chapter, provisions specific to Germany as a Member State to international agreements such as CITES and the CBD as well as Member State to the European Union will be analyzed.

I. General Information on the German environmental law

In 1994, the principles of sustainability and the protection of natural resources and animals were added to the German constitution.[228] The underlying article is a state objective with a legally binding impact and could be classified as directive for governmental action.[229] The state objective represents the long-term aim of sustainable development and the precautionary principle determined by the Rio-Declaration.[230] The two most important national legislative acts concerning the conservation of species and environment in German law are the “Federal Nature Conservation Act” (Bundesnaturschutzgesetz)[231] which is complemented by the “Federal Species Protection Regulation” (Bundesartenschutzverordnung).[232] The “Federal Species Protection Regulation” enhanced the number of strict protected species under the “Federal Nature Conservation Act”[233] and

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[227] Art. 114, *TFEU*; *Sparwasser/ Engel/ Voßkuhle*, Umweltrecht- Grundzüge des öffentlichen Umweltschutzrechts, C.F. Müller Lehr- und Handbuch (5, 2003), 44.

[228] Art. 20a II GG; Grundgesetz für die Bundesrepublik Deutschland in force 23 May 1949, last amendment 11.07.2012 (BGBl. I S. 1478).

[229] *Bundesminister des Innern und der Justiz (BMI/BMJ) (Hrsg.),* Bericht der Sachverständigenkommission „Staatszielbestimmungen/Gesetzgebungsaufträge“, 1983, 360ff.

[230] Rio Declaration on Environment and Development, *UN*, UN Doc. A/CONF.151/26 (vol. I); 31 ILM 874 (1992) (Rio Declaration); *See also: Calliess,* Rechtsstaat und Wirtschaftsfreiheit vor den Herausforderungen des Staatsziels Umweltschutz (Art. 20a GG) (Rule of Law, Freedom of Market and Environmental Protection), Berlin e-Working papers on European law, (2004), 8.

[231] *Bundesnaturschutzgesetz (BNatSchG*) in force 1 March 2010 (BGBl. I, 2542), last amendment 7 August 2013 (BGBl. I, 3154).

[232] *Bundesartenschutzverordnung (BArtSchV)* in force 16.02.2005 (BGBl. I, 258, 896), last amendment 21.01.2013 (BGBl. I S. 95).

[233] *BNatSchG,* § 10 (2) (10, 11).

supplemented the protection instruments.[234] However, also other regulations and legislation contain legal regulations on species protection such as the “Federal Game Law” (Bundesjagdgesetz) and the “Federal Wildlife Regulation” (Bundeswildschutzverordnung).

II. German environmental policy and the role of hybrids

Due to the fact that the European Regulation on “the protection of species of wild fauna and flora by regulating trade therein” (Commission Regulation (EU) 750/2013) is directly legally binding in German national law, the implementation of CITES and especially of the Conference Resolution 10.17 on animal hybrids, generally non-legally binding, is also indirectly transposed into German national law. The guidance document “Vollzugshinweise zum Artenschutzrecht”[235] provides guidance for the correct implementation of the different legal sources. The guidance document points out that the provisions for hybrids differ with regard to the applicable law. Thus, if a species falls within the scope of the European Wildlife Trade Regulation, because it is listed in one of the respective annexes, then a hybrid of this species obtains the same conservation status as the parental species. This applies to hybrids until the fourth generation.[236] However, the regulations differ if hybrids only fall within the scope of national law. The “Federal Species Protection Regulation” elucidated in Appendix 1 to Paragraph 1 that ‘bastards’ of species (i.e. hybrids), listed in Appendix one, should obtain the same legal status as the parental species.[237] The Federal guidance document, however, demonstrates that only hybrids with at least one purebred parental species fall within the scope of protection. Second generation hybrids (i.e. offspring of two hybrids) are not considered by national law.[238]

In accordance with the provisions of the “Federal Species Protection Regulation” and for the purpose of the “Federal Nature Conservation Act” (§§7, 44) all native amphibian species are protected in Germany. Whether the hybrid frog *Pelophylax esculentus* can be considered a “native amphibian species” is debatable.[239] However, it is listed under its former name ‘*Rana kl. esculenta*’ in the non-legally binding

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[234] *BNatSchG*, § 42 et seq.

[235] *LANA- Länderarbeitsgemeinschaft Naturschutz*, Vollzugshinweise zum Artenschutzrecht (12-13 March 2009). A mandatory application for a consistent enforcement is recommended by the “environment minister conference“ (Umweltministerkonferenz) (Umlaufbeschluss Nr. 23/2007).

[236] *Ibid.*; 28-29.

[237] Appendix 1 (to § 1), Schutzstatus wild lebender Tier- und Pflanzenarten, Erläuterungen zur Anlage 1 (4), *BArtSchV*, (Comments No. 4 to enclosure 1), (BGBl. I 2005, 264 -285).

[238] *LANA- Länderarbeitsgemeinschaft Naturschutz*, Vollzugshinweise zum Artenschutzrecht (12-13 March 2009), 28-29.

[239] *See* C. III.

Red List of Threatened Species in Germany which provide guidance for policy- and decision-makers in order to ensure effective nature conservation.[240] The species name used in the Red List is the only indication that this taxon is a hybrid as the abbreviation 'kl.' represents the abovementioned species category "klepton" for clonal and hemiclonal hybrids. It can thus be concluded that this particular form of a hybrid is recognized in German nature conservation as a distinct species or at least it can be managed as such for purposes of conservation. Therefore, it needs to be protected under German national law following §§7, 44 BNatSchG.

The "Rackelwild" (*Lyrurus tetrix* × *Tetrao urogallus*) is a bird hybrid and represents another hybrid taxon regulated under German national law. In contrast to the hemiclonal frog, this taxon represents a typical hybrid form with an admixed genome (the cross between the parental species names indicates the hybrid status). Although it occurs only rarely in nature[241] and does not form stable populations, this hybrid is listed in Appendix 1 to § 2 (1) of the "Federal Wildlife Regulation" and is subject to the "Federal Game Law" (§ 2 (1)). The same applies to both parental species which, moreover, are strictly protected under the "Federal Species Protection Regulation".[242] This suggests that the inclusion of this hybrid taxon in the two appendices is attributable to the protection of both parental species. In order to prevent illegal killing, capture or trade of the parental species, a conservation status for naturally occurring hybrids represents an appropriate measure. Considering that in this case the hybrid is explicitly mentioned in the legal texts of both legislations, it is assumed that their provisions apply to all generations, even though this is not defined in any of them.

In addition, a separate regulation for raptor hybrids in the "Federal Species Protection regulation" is in place since 2005.[243] According to this regulation, breeding and keeping of raptor hybrids is prohibited as well as free flight of hybrids in the wild. The background for this revision was pointed out by an official explanation[244] stating that strict

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[240] *Bundesamt für Naturschutz*, Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands, LV Druck GmbH & Co. KG, Münster (2009): 1-386, 266. Red Lists assess the conservation status of species and are used for the coordination of national and international nature conservation. They present a non-legally binding information source for legislative measures; www.bfn.de/0322_rote_liste.html, last accessed March 2015.

[241] *Höglund/ Porkert*, Experimentelle Kreuzungen zwischen Auer-und Birkhuhn (Tetrao urogallus et Tetrao tetrix), Zeitschrift für Jagdwissenschaft 35.4 (1989): 221-234, 229.

[242] *BArtSchV*, (Comments No. 4 to enclosure 1), (BGBl. I 2005, 264 -285).

[243] *BArtSchV*, §§ 8-11. In force 25 February 2005 (BGB1. I 2005, 258). For the purpose of this regulation raptor hybrids are defined as individuals with genetic shares of at least one native raptor species and one other raptor species.

[244] BR-Drs. 800/04 (22 October 2004), Regulation of the *Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (BMU)* and the *Bundesministeriums für Verbraucherschutz, Ernährung und Landwirtschaft (BMVEL).*

provisions dealing with raptor hybrids are necessary to ensure the survival of native raptor species. Raptor hybrids jeopardize the population and may hamper the recolonization of native species and compete for food and reproduction. Furthermore, fertile hybrids could introduce non-native genes in the wild native population, which may change the adaptive diversity of the population and may result in a shift of the whole ecosystem.[245] However, options for action in relation to wild-born raptor hybrids or astray hybrids are not considered under this regulation. Thus, it can be concluded that it represents more a regulation with a precautionary approach to avoid hybridization processes in the wild. A similar approach for hybrids of other species does not exist in German national law.

As Member State to the European Union, Germany has to implement the Bern Convention and the Habitats Directive into national law. Paragraph 44 et seq. of the "Federal Nature Conservation Act"[246] obliged the 16 Federal states to meet the requirements given by the Article 12 of the Habitats Directive.[247] One example for the implementation of both legal instruments in Germany is exemplified by the wolf (*Canis lupus*). The wolf is a strictly protected species under German national law (§§7, 44, 45 BNatSchG) and also under European law (Annex II and IV Habitats Directive). Furthermore, it is listed in Appendix II of CITES and annex II of the Bern Convention. In order to ensure the protection of wolf populations in Germany, most of the Federal states present management plans[248], which are adapted to the European Guidelines[249] and the German Guidelines[250] for wolf management. With regard to species conservation these guidelines propose an immediate removal of hybrids from nature[251] which is implemented by the States management plans.[252] However, in face of potential conflicts with hunters and owners of livestock both guidelines as well as the management plans request the same legal status for wolf-dog hybrids as for pure wolves.[253] In those Federal states where wolf management plans already exist, the removal of hybrids is strictly regulated. The removal is performed only by experts and only if the hybrid status is genetically confirmed.

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[245] *Ibid.*, 111.

[246] § 32 (1-3) *BNatSchG.*

[247] Art. 12, *Habitats Directive,* (OJ 1992 L206/12).

[248] http://www.nabu.de/wolf/wolfsmanagement.jpg, last accessed September 2014.

[249] *Linell/ Salvatori/ Boitani,* (2008).

[250] *Reinhardt/ Kluth* , Leben mit Wölfen: Leitfaden für den Umgang mit einer konfliktträchtigen Tierart in Deutschland, Bundesamt für Naturschutz (BfN-Skripten 201/2007) (FKZ 805 86 007).

[251] *Linell/ Salvatori/ Boitani,* (2008): 77- 78; *Reinhardt/Kluth*, BfN-Skripten 201 (2007): 105.

[252] For example: Managementplan für den Wolf in Mecklenburg- Vorpommern, *Ministerium für Landwirtschaft, Umwelt und Verbraucherschutz Mecklenburg-Vorpommern* (July 2010), 21. Managementplan für den Wolf in Sachsen, *Sächsisches Staatsministerium für Umwelt und Landwirtschaft* (May 2008), 25.

[253] *Ibid.*; *Linell/ Salvatori /Boitani,* (2008): 78; *Reinhardt/Kluth,* BfN-Skripten 201 (2007): 108.

Moreover, an exception permit issued by the pertinent authority is necessary for a legal removal or killing of hybrids. The German Guidelines favor a lethal control over captivity for animal welfare reasons.[254] The rationale for this is based on a case example in which the behavior of two captured hybrids has shown that a life in captivity could be a torture and contradicts the ideas of animal welfare.[255] Due to the fact that stray dogs are rare in Germany the risk of hybridization with dogs appears to be minor.[256] However, the German Guidelines endorsed precautionary measures such as telemetry, particularly of female wolves. Moreover, the Guidelines recommend citizens to look after their dogs particularly during the mating season of wolves to prevent interspecific matings.[257] Another important part in all management plans and guidelines is the improvement of public awareness concerning hybridization.

The German non-governmental nature conservation organization (NABU) suggested on its webpage[258] an amendment of the Federal Game Law concerning poaching dogs. Under special circumstances hunters are allowed to kill stray cats and dogs if they are killing game.[259] The NABU criticizes that this article could be used by hunters to justify illegal killing of wolves as mistaking them with a straying dog. This argument has recently been used during the court case on an illegal wolf killing in the Westerwald region in Rhineland Palatinate in Germany.[260] The accused hunter asserted that he mistook the wolf as a stray dog when shooting it. Genetic analyses confirmed that the specimen was a purebred wolf which had immigrated from Italy. The hunter was convicted with a financial penalty of 3500,-- €. The NABU recommends special exception permits for the removal or lethal control of stray dogs. Considering that hybrids are hard to distinguish morphologically from wolves, mistaking hybrids and wolves by hunters could not be excluded. Thus, legalizing the hunt of hybrids would increase the risk of illegal wolf killing in face of mistaking them with hybrids.

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[254] Managementplan für den Wolf in Mecklenburg- Vorpommern, *Ministerium für Landwirtschaft, Umwelt und Verbraucherschutz Mecklenburg-Vorpommern* (July 2010), 21-22.

[255] *Reinhardt/Kluth,* BfN-Skripten 201 (2007): 105-106.

[256] *Ibid.*, 105-107.

[257] *Ibid.*, 107.

[258] Naturschutzbund Deutschland e. V. is a non-governmental organization with the aim of the correct implementation and improvement of nature conservation. The NABU supports Germany and the Federal states, *inter alia,* in proposals for regulations on nature conservation. *See also*: http://www.nabu.de/aktionenundprojekte/wolf/jagdundverfolgung/14786.html, Comment: Mutmaßlicher Täter stellt sich, last accessed November 2014.

[259] § 23, *BJagdG.*

[260] Pressemeldung *Amtsgericht Montabaur*, Strafprozess um getöteten Wolf im Westerwald (17.01.2013), http://www.mjv.rlp.de/icc/justiz/nav/634/broker.jsp?uMen=634b834c-d698-11d4-a73d-0050045687ab&uCon=f7d60fbd-61b8-4c31-4f1b-f33577fe9e30&uTem=aaaaaaaa-aaaa-aaaa-aaaa-000000000042, last accessed September 2014. The district court (Amtsgericht) represents one part of the ordinary jurisdiction in Germany. *See also* Art. 92, 97 GG and §24 Gerichtsverfassungsgesetz (GVG).

III. Preliminary Conclusion

A legal binding definition of hybrids and hybridization is absent also in German national law. As Member State to CITES and the European Union, Germany has to implement their regulations, including any provision on hybrids. Hybrids receive the same conservation status as the protected parental species until the fourth generation. However, there is an exception if the included species falls only under the scope of German national law. In this case, only hybrids of the first generation obtain the same conservation status as their parental species. Hybrids of further generations do not fall under the scope of the national law. The situation is different when hybrid taxa are explicitly mentioned in annexes as in the case of *Pelophylax esculentus*[261] and *Lyrurus tetrix* × *Tetrao urogallus*. In these cases hybrid taxa obtain the same conservation status as their purebred relatives independent of the generation. Furthermore, Germany has adopted a special regulation on raptor hybrids which follows the precautionary principle. This separate regulation reveals that in special situations a case-by-case handling is necessary. Concerning the implementation of the Habitats Directive, the relevant Federal states implement different management plans for the conservation of wolves including strategies for dealing with hybrids and prevent hybridization. The example `wolf´ shows that hybrids should receive the same legal status as the protected parental species to avoid a loophole for illegal killing of purebred individuals even if hybrids present a risk for the population. However, statutorily regulated removal of hybrids from nature is necessary in species conservation to avoid genetic displacement processes. The German perspective in relation to the way of removal is interesting, as the German Guidelines for wolves favors a lethal control of hybrids over captivity to ensure animal welfare. Captivity is seen as a torture for wild wolf-dog hybrids and unacceptable in animal protection. However, it must be noted that such an approach may not be appropriate in every situation when dealing with hybrids.

E. Conclusion and summary

Natural hybridization is an important part of evolution and is necessary for the existence of biodiversity. In contrast to natural hybridization, anthropogenic hybridization represents a threat to biodiversity. The possible worst case scenario given by scientific experts reveals that anthropogenic hybridization could lead to local or even complete extinction of species

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[261] However, it must be regarded that distinguishing the generation in a hemiclonal hybrid such as *Pelophylax esculentus* is scarcely possible as hybrid descendants have in general the genome of a first generation hybrid. Only triploid individuals may indicate the second generation of this taxon.

by genetic displacement.[262] Especially rare species are threatened. However, the problem appears to be relatively new compared to other anthropogenic disturbances like habitat modifications, climate change and invasive species, but it slowly obtains increased attention in nature conservation. The final conclusion of this study provides an overview of the role of hybrids and hybridization in the three analyzed levels of law. Existing legal norms are pointed out as well as the existing difficulties and challenges when dealing with hybrids and hybridization. Finally, possible opportunities for the management of hybrids and hybridization in a conservation perspective and their necessity are discussed.

Terminology

The term 'hybrid' is used in international and European environmental law, however, it is not consistent according to all fora. Even as States party to conventions such as CITES and as a Member State to the European Union, Germany uses additionally to the term 'hybrid' the old term \`bastard´.[263] On the one hand, this word is an old-fashioned synonym to hybrid. On the other hand, this term has a negative connotation in Germany, where it is used colloquial as swear word for a perceived inferior person.[264] Another example is used by the United States of America (USA).[265] In the USA the term \`intercross´ is used synonymously in a policy on hybrids due to related negative connotations associated with the term 'hybrid'.[266] Despite of negative connotations with some concepts, it is important for a consistent understanding and application to agree upon a single term. Any discordance concerning terminology presents a legal uncertainty. The missing clarity could lead to different interpretations and definitions. Depending on the protection target, hybridization and hybrids may be interpreted positively or negatively. In order to gain public attention for a conservation aim, the association with the term is important and

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[262] *Rhymer/ Simberloff,* Annual Review of Ecology and Systematics (1996): 83-109, 89.

[263] *BArtSchV,* Erläuterungen Nr.4 zur Anlage 1 (Comments No. 4 to enclosure 1), (BGBl. I 2005, 264 -285); *LANA- Länderarbeitsgemeinschaft Naturschutz,* Vollzugshinweise zum Artenschutzrecht (12-13 March 2009), 28-29.

[264] The definition is presented by the German spelling dictionary. „Meaning: 1a. nicht eheliches Kind besonders eines Adligen und einer nicht standesgemäßen Frau“ (illegitimate child of a nobleman and a socially unacceptable woman), „1b. als minderwertig empfundener Mensch (auch als Schimpfwort)“, (swear word for a perceived inferior person)*;* http://www.duden.de/rechtschreibung/Bastard, last accessed November 2014.

[265] The American national law presents an independent strategy on dealing with hybrids and an own policy. But based on the complexity, the study is restricted on a European example.

[266] *Federal Register* (1996) Endangered and threatened wildlife and plants; proposed rule on the treatment of intercrosses and intercross progeny (the issue of 'hybridization'); Request for public comment. Federal Register 61, No. 26, 4710-4713, 4710; “Intercross and Intercross Progeny Defined. Due to connotations attached to the various terms that are in general use for matings across taxonomic boundaries and for their products (e.g., cross, hybrid, intergrade, and interbreed), the Services propose to use the neutral term \`\`intercross'' for all crosses between individuals of different”.

should not be influenced otherwise. However, the terms ‘hybrid’ and ‘hybridization’ are scientific terms which are already established in three very important international conventions on environmental conservation (CITES/ CBD/ Bern Convention) as well as in European environmental law. Thus, the retention of the terms is recommended. More importantly, however, is a consistent definition.

Definition

The present study demonstrates that one major problem in setting conservation guidelines is the definition of the terms ‘hybrid’ and ‘hybridization’. A legally binding definition is missing in all three analyzed levels of law. Although the term ‘hybridization’ is used in some of the analyzed documents, a separate definition apart from the term ‘hybrid’ is only found in the non-binding IUCN glossary.[267] Certainly, the non-legally binding definitions proposed by CITES and the German national law include the emergence of a hybrid animal in its definition for ‘hybrid’.[268] In this case, hybrids are the progeny of two different species. However, it must be considered that hybridization does not necessarily lead to a mixed gene pool. Hybrid offspring can be absent due to reproductive interference[269] or the F1-generation may be infertile.[270] In these cases, hybridization still has a (even lager) negative impact on the fitness of a species, which is not always obvious.[271] It is assumed that the absence of a legally binding definition of hybridization is a consequence of the discordance of scientific experts and the widely discussed species concepts.[272] It remains unclear if the mating process of a hybrid and a pure species can be counted as hybridization, since it is not the mating of two completely different species. This is also the case when two hybrids mate. Nonetheless, these mating processes can result in a genetically mixed offspring. Additionally, a differentiation of natural and anthropogenic hybridization in a legal sense is necessary for a legal assessment of hybridization.

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[267] *IUCN*, glossary: Hybridization is defined as “a cross-breeding between individuals of different species”, http://cmsdata.iucn.org/downloads/en_iucn__glossary_definitions.pdf, 34, last accessed November 2014. It represents a non-binding definition.

[268] *CITES*, glossary: A hybrid is defined as “an animal or plant that is produced as the result of cross-breeding between two different species.”, http://cites.org/eng/resources/terms/glossary.php#h, last accessed November 2014. *LANA-Länderarbeits-gemeinschaft Naturschutz*, Vollzugshinweise zum Artenschutzrecht (12-13 March 2009), 28-29, 28. “Hybride (lat. Hybrida: der Bastard) entstehen durch die Kreuzung verschiedener Arten oder Unterarten.“

[269] *Hochkirch*, Journal of Evolutionary Biology 26.2 (2013): 247-251, 247. “Def. Reproductive interference: Genetic incompatibilities during fertilization or embryonic development, which avoid hybrid offspring”.

[270] *Allendorf/ Leary/ Spurell/ Wenburg*, Trends in Ecology & Evolution 16.11 (2001): 613-622, 616.

[271] *Gröning/ Hochkirch* (2008) Reproductive interference between animal species, The Quarterly Review of Biology 83: 257-282, 257. *Rhymer/ Simberloff*, Annual Review of Ecology and Systematics (1996): 83-109, 84.

[272] *Harrison*, in *Richard G. Harrison*, Hybrid Zones and The Evolutionary Process (1993) 3-12, 4-6. *See also* A. *Definition*.

According to the aforementioned definition endorsed by CITES, an animal is a hybrid if the parents belong to two different species. This would mean that only hybrids of the first generation are hybrids under this definition, whereas descendants of two hybrids as well as backcrosses are not counted as a hybrid. This issue was considered by CITES in the corresponding Resolution Conference 10.17 on Animal Hybrids. In pursuance of this resolution, CITES proposed to interpret the term ‘hybrid’ as the previous four generations.[273] This recommendation was adopted by the European environmental law and the German national law. This holds at least for hybrids which have no less than one parental species listed in one of the annexes of CITES or the European Union Wildlife Trade Regulation in its lineage.[274] In its national law, Germany uses an own definition for hybrids of species that do not fall under the scope of the abovementioned regulations. In this case, only hybrids of the first generation are counted as hybrids and achieve a conservation status under German national law. This example underlines the uncertainty and discordance in this section of law and reflects the scientific uncertainty mentioned in section I.

Development of the inclusion of hybrids in a legal framework and the legal classification today

International environmental law

The first resolution on Animal hybrids was submitted by CITES in 1979. The main aim of this convention is to prevent wild species from overexploitation by international trade. In light of hybrids, the Animal Committee to CITES recognized the problem of distinguishing hybrids from purebred species and warns of a possible gap in the law. A non-legally binding resolution on hybrids was adopted by the States parties to CITES in order to prevent that trading entities declare purebred species as hybrids to circumvent trade regulations. Furthermore, another non-legally binding resolution enables the inclusion of hybrids in the appendices of CITES if they form stable populations in the wild. Interestingly, hybrids are regulated in trade law, but such a separate provision in environmental law and the corresponding agreements affecting biodiversity is missing.

The CBD refers to the threats to biodiversity naming also the three main threats supporting hybridization (invasive alien species, climate change and habitat destruction)[275], however, the term ‘hybridization’ first appeared in 2001 in relation to invasive species

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[273] *CITES* Resolution Conf. 10.17 (Rev. CoP14).

[274] *European Commission*, Council Regulation (EC) No 338/97 (OJ 1997 L 61,1).

[275] *Secretariat of the CBD* (2010) Global Biodiversity Outlook 3, 9.

without a definition.[276] Although the problem of genetic displacement of native species by invasive species (including released or escaped domesticated and captive bred species)[277] was recognized, a decision how to deal with hybrids is missing. A recommendation published by the SBSTTA provides guidance for a risk assessment of invasive species as pets or the like, thereby, the SBSSTA includes hybrids as invasive species.[278] Thus, it now depends on the COP to the CBD whether and how hybrids are included in a, however, non-legally binding decision on invasive species. In 2006, the COP to the CBD requested in their Decision on Island Biodiversity the development of science-based risk assessment methodologies including hybridization as potential threat.[279] Furthermore, in 2010 the risk of climate change to affect species interactions was pointed out by the Parties to the CBD.[280] Although, there is no regulation how to deal with hybrids in the wild, the mentioned approaches show that the Parties to the most important convention relating to biodiversity started to recognize the problem and proposed measures to prevent hybridization by regulating invasive species and climate change. The analysis of the CBD approach towards hybridization has shown that a significant degree of scientific uncertainty exists which appears to restrain the CBD from a concrete regulation on hybrids.

The Bern Convention is of specific importance for the European wildlife. Although the phenomenon of hybridization with invasive and domesticated species is recognized by the Standing Committee to the Bern Convention, only two non-legally binding recommendations are available. Both approaches are species-specific and recommend the removal of hybrids from the wild. The first recommendation requests the total eradication of hybrids with invasive species without any restrictions.[281] In contrast, the Standing Committee proposes in the second recommendation a controlled removal of hybrids with domesticated species only if the identification is ensured.[282] Furthermore, a prohibition to keep wolves and wolf-dog hybrids as pets is suggested to minimize the probability of hybridization with its wild congener. Interestingly, the recommendation was published fourteen years after the threat of hybrids for wild wolf populations was first recognized by the Group of Experts on Conservation of Large Carnivores as noted in the Action Plan for

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[276] UNEP/CBD/SBSTTA/6/INF/11, 7, *SBSTTA*, (Montreal, Canada, 1-10 March 2001).
[277] UNEP/CBD/COP/DEC/XI/28, *CBD*, (Hyderabad, India, 8-19 October 2012).
[278] *See* B. III. Hybridization in the context of CBD.
[279] UNEP/CBD/COP/DEC/VIII/1, *CBD*, (Curitiba, Brazil, 20-31 March 2006).
[280] UNEP/CBD/SBSTTA/14/INF/21, 13, *SBSTTA*, (Nairobi, Kenya, 10-21 March 2010).
[281] Council of Europe, *Standing Committee*, Recommendation No. 149 (2010).
[282] Council of Europe, *Standing Committee*, Recommendation No. 173 (2014).

Wolves.[283]. This long period appears to have been caused by the significant amount of scientific uncertainty and the development of new techniques to detect hybrids. However, these approaches present useful examples of how to deal with hybrids of native and non-native species and therefore, provide a suitable basis for international and national legislation on hybrids. In order to avoid illegal killing and overexploitation of species, the conservation status of hybrids provided by CITES and the government-controlled removal of hybrids recommended by the Bern Convention appears to be an adequate solution.

European environmental law

The analysis of the European environmental law concerning the treatment of hybrids reveals that beside the European Union Wildlife Trade Regulation and the regulation on invasive alien species no other regulation is dealing with hybridization or hybrids, not even the Habitats Directive. However, a single hybrid taxon is listed in Annex V to the Habitats Directive as it forms stable populations in the wild and does not represent a threat to its purebred congeners. The recently adopted regulation on invasive alien species[284] recognizes the threat of "genetic effects by hybridisation" and presents a definition for the term `alien species´ which also includes hybrids of native and invasive species. As well as the LCIE the regulation urges a removal of hybrids with invasive species from the wild. Notwithstanding, the absence of a concrete legally binding definition of the term 'hybrid' and its generation this represents the first legally binding regulation dealing with wild-born hybrids.

German national law

The German national law presents its own regulation on raptor hybrids and prohibits the breeding and keeping of raptor hybrids since 2005 to prevent native raptor populations against negative genetic effects. In addition, two hybrid taxa are regulated under German national law and obtain the same conservation status as their purebred relatives. However, it is not uniquely defined up to which generation hybrids fall within the scope of the respective legislation. Furthermore, German Federal states are oriented towards the European guidelines of the LCIE concerning the handling of wolf-dog hybrids, whereby a lethal control of hybrids by authorized people is favored.

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[283] Council of Europe, *Group of Experts*, established by *Boitani*, (2000) on Conservation of Large Carnivores - Action Plan for the conservation of the wolves (*Canis lupus*) in Europe, (T-PVS (2000) 23).

[284] *European Parliament and the Council of the European Union*, Regulation (EU) No. 1143/2014 of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. (OJ 2014 L317/35-55), entered into force on the 01.01.2015 (Article 33).

Meaning and possibilities

Protection or control of hybrids

A successful policy on hybrids and hybridization depends on a consistent terminology and definition.[285] Thus, provisions dealing with biodiversity need to include a general legal definition which could be broadened in a species-specific context. Although anthropogenic causes for hybridization present the main threat to biodiversity, natural causes should also be considered, as a strict separation of causes for hybridization processes is not always possible.[286] Climate change for example could be induced by anthropogenic and/or natural conditions. In both cases, it can alter biodiversity compositions and could facilitate hybridization processes.

Another important aspect to consider is that beside the regulations on invasive species, only species that are listed in the respective annexes are considered by law, whereas hybrids of non-listed species are neglected. However, hybridization could affect the conservation status of any species independent of its representation on the annexes. If new hybridization processes emerge, it would be useful to monitor these processes to analyze the causes and consequences of hybridization and assess the level of threat caused by it. A register of hybridization could help to focus research in response to emerging threats and develop strategic measures for its conservation.

Prevention

Precautionary measures to prevent the introduction or spread of invasive species, climate change and habitat destruction as presented by the treaties dealing with biodiversity and species trade indirectly also help to reduce the risk of hybridization. Although hybridization is mainly mentioned incidentally in all these approaches at international, European and national level, it is essential to specify these precautionary measures to prevent biodiversity loss. Remaining purebred populations of species, threatened by hybridization in other parts of its range, need to obtain a priority status in conservation. Moreover, building public awareness is of high importance to make the citizens aware of these problems, particularly if domesticated or traded species are involved. In order to minimize the risk of hybridization with domestic animals, uncontrollable free ranging specimens (e.g. cats or dogs) should be sterilized if they share an overlapping territory with their wild relatives. Additionally, in cases where it can be problematic to ensure the

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[285] *Trouwborst*, RECIEL 23.1 (2014): 111-124, 110; *Allendorf/ Leary/ Spurell/ Wenburg*, Trends in Ecology & Evolution 16.11 (2001): 613-622, 613.

[286] *Ibid.*, 613.

conservation of native biodiversity, the keeping of wild species and their hybrids should be prohibited.[287]

Protection

The effects of hybridization on a population of a species are not always negative. In nature, hybridization is also an evolutionary process, which can facilitate the adaption to new environmental conditions. As mentioned above, the boundaries between natural and anthropogenic hybridization can be smooth. Climate change could be caused by anthropogenic or natural influences and lead to environmental changes which can result in hybridization of two naturally distinct species. An adaptation to the new environmental conditions may be necessary to ensure the survival of a species, even though its current genetic integrity is lost.[288] Consequently, a new species may emerge, but it may also go extinct. An example for hybridization of two species facilitated by climate change is illustrated by the polar bear and the grizzly bear.[289] The melting arctic ice, a consequence of climate warming, removes the natural geographic barrier between both species and leads to overlapping habitats. Therefore, an increasing number of hybrids of both species, so called `Pizzlies´ or `Grolar Bears´, are found in the natural habitat. *Kelly et al.* assume that:

> If polar bears survive climate change in secluded refuges - which is far from certain - interbreeding could be the final straw.

In the case of the polar bear, hybridization can be considered as an evolutionary process in the face of anthropogenic climate change. Some authors fear that with the melting of arctic ice a survival of the species *Ursus maritimus* (polar bear) is highly improbable.[290] An ongoing hybridization with grizzly bears could ensure a new adaptation to the changing habitat and retain polar bear specific genes. Furthermore, hybridization could generate a new species, but this remains unpredictable. However, hybridization could also weaken a population if hybrids are less fit or infertile. Taking into account the existing risk of extinction independent of hybridization, the negative impact of hybridization appears to be low. Considering the unpredictable future of the arctic, a

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[287] Council of Europe, *Standing Committee*, prepared by *Trouwborst, A.*, (T-PVS/Inf (2014) 15).

[288] *Chunco*, Ecology and Evolution 4.10 (2014): 2019-2031, 2026; *Barton*, The role of hybridization in evolution, Molecular Ecology 10.3 (2001): 551-568, 551.

[289] *Kelly/ Whiteley/ Tallmon,* Nature 468.7326 (2010): 891.

[290] *Derocher/ Lunn/ Stirling.*, Polar bears in a warming climate. Integrative and Comparative Biology 44.2 (2004): 163-176, 163; *Schliebe/ Derocher/ Lunn* (IUCN SSC Polar Bear Specialist Group), 2008, *Ursus maritimus*. The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org, last accessed September 2014.

human intervention, i.e. the removal of hybrids from the wild, does not appear useful. The polar bear, as all other *Ursus* species, is listed in the Appendix II of CITES. This means that the hybrids are also protected under this convention. In order to protect *Ursus maritimus* this trade regulation is useful and necessary to avoid illegal trade and killing. Besides this regulation, the only suitable measure to protect this species and prevent hybridization is to prevent ongoing climate warming. This example shows that if the causes for hybridization are natural, not guidable or controllable such as climate change, hybridization becomes a part of an anthropogenic evolutionary future, which may be observed and documented but not intervened by humans. Nonetheless, the conservation status for wild-born hybrids as presented by CITES is necessary to avoid loopholes for trading entities and hunters. Only with this level of protection the establishment of hybrids and the formation of a new species are possible.[291] If hybrids already form stable populations apart from the parent taxa, it is quite useful to include them as hybrid taxon or species in the respective annexes of the provisions in order to ensure their survival as in the case of *Pelophylax esculentus*.[292]

Combating hybridization and hybrids

As mentioned before, a conservation status for hybrids is necessary, particularly in trade and game law. Nonetheless, native populations may be threatened by the introduction of invasive and domesticated species due to hybridization. Unlike climate change, the opportunities for action in terms of invasive species are manifold. It needs to be distinguished whether a few specimens of a population are hybrids or if a large part of the global population of a species shows a widespread or complete introgression. Controlling invasive species by removing them or their hybrids from the wild could be a suitable management method, if introgression is limited. In these cases, the genetic identification of hybrids is necessary to avoid wrong removals, which may weaken the natural population and thus fail the conservation aim. A removal could be done by lethal control or accommodation in captivity and should be decided in a species-specific approach.[293] Furthermore, a removal should only be performed by authorized persons possessing a special permit to avoid misuse. The genetic identification of hybrids is species-specific and the methods need to be developed or improved by the corresponding experts. However, a removal of hybrids is only recommended, if the threatened species involved could, in that

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[291] *Allendorf/ Leary/ Spruell/ Wenburg*, Trends in Ecology & Evolution 16.11 (2001): 613-622, 618.

[292] *See also* C. III.

[293] Council of Europe, *Standing Committee*, prepared by *Trouwborst, A.*, (T-PVS/Inf (2014) 15).

way, be protected against genetic introgression. Furthermore, such measures are only applicable to large animals like large mammals or raptors, which are easier to monitor by humans or to species with small population sizes. The control of small animals with high reproductive rates is not feasible and any counteraction such as removing single specimen is unlikely to solve the problem. *Allendorf et al.* presents a categorization of hybridization including possible conservation perspectives, if complete populations of a species are affected by hybridization.[294] Regarding anthropogenic hybridization the authors distinguish three types of hybridization. In the case of hybridization without introgression (F1-generation hybrids are sterile), they recommend a removal of the invasive species and the F1-generation hybrids. In cases of hybridization with widespread introgression, a maintaining and expanding of the remaining purebred populations is assumed to be effective for the conservation of the threatened species. As a third type of hybridization the authors name the complete introgression of all populations. In this case, the protection of hybrids is claimed "in hope that they will fill the ecological role of the native taxon".[295]

Even if other causes play a major role and contribute to hybridization, the present study reveals that a consistent terminology and definition must be provided by the respective international treaties and implemented by national laws. Moreover, legally and non-legally binding provisions dealing with invasive species, habitat loss or climate change need to include measures on hybridization and hybrids. Based on the complexity of this topic and its different causes a general answer to the question: `How to deal with hybrids and hybridization?´ is impossible. Precautionary measures, depending on the causes of hybridization, are of crucial importance to prevent hybridization. However, a species-specific approach (e.g. wolf-dog hybrids, raptor hybrids, edible frog and "Rackelwild") presents the only possible way for an efficient handling of hybrids.[296] Thus, a guideline how to deal with hybrids under certain circumstances may provide an appropriate assistance for decision-makers. However, besides the concept of conservation it must be considered that hybridization may also represent a natural evolutionary process of adaptation of species to changing environmental conditions, even if it finally may result in extinction of a species.

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[294] *Allendorf/ Leary/ Spruell/ Wenburg*, Trends in Ecology & Evolution 16.11 (2001): 613-622, 616.

[295] *Ibid*., 618.

[296] *Björklund*, Journal of Evolutionary Biology 26 (2013), 274-275, 275; "A hybridization event is totally dependent on the initial conditions and can therefore be predictable only if we know all the details of the hybridizing individuals and the ecological conditions at hybridization"; Council of Europe, *Standing Committee*, prepared by *Trouwborst, A.*, (T-PVS/Inf (2014) 15).

Summary

Climate change and habitat fragmentation modify the natural habitat of many wetland biota and lead to new compositions of biodiversity in these ecosystems. While the direct effects of climate are often well known, indirect effects due to biotic interactions remain poorly understood. The water meadow grasshopper, *Chorthippus montanus*, is a univoltine habitat specialist, which is adapted to permanently moist habitats. Land use change and drainage led to highly fragmented populations of this generally flightless species. In large parts of the Palaearctic *Ch. montanus* occurs sympatrically with its widespread congener, the meadow grasshopper *Chorthippus parallelus.* Due to their close relationship and their similar songs, hybridization is likely to occur in syntopic populations. Such a species pair of a habitat specialist and a habitat generalist represents an ideal model system to examine the role of ongoing climate change and an accumulation of extreme climatic events on the life history strategies, population dynamics and inter-specific interactions. Therefore a multiple method approach (laboratory experiments, population genetics, population ecology, mark-recapture-studies, legal assessment) was performed in this thesis.

In **Chapter I** a laboratory experiment was conducted to identify the impact of environmental factors (temperature and density) on intra-specific life-history traits of *Ch. montanus*. As this species is highly immobile, the initial aim of this study was to verify the hypothesis that high densities or temperatures induce an increasing rate of macropterism which may increase the dispersal possibilities of this species. Although this hypothesis was not confirmed, an impact of density and temperature on the life history traits was observed. Like other Orthoptera species, *Ch. montanus* follows a converse temperature size rule (increasing body size with higher temperatures). In line with the dimorphic niche hypothesis, which states that sexual size dimorphism evolved in response to the different sexual reproductive roles, both sexes showed different responses to increasing density at lower temperatures. Males attained smaller body sizes at high densities, whereas females had a prolonged development time. This is the first evidence for a sex-specific phenotypic plasticity in *Ch. montanus.* Females benefit from the prolonged development as their reproductive success depends on the size and number of egg clutches they may produce. By contrast, the reproductive success of males depends on the chance to fertilize virgin females, which increases with faster development. This may become a disadvantage for *Ch. montanus* as an intraspecific phenology shift may increase hybridization risk with the sibling species.

Despite the widespread assumption that hybridization between two sympatric species is rare due to complete reproductive barriers, the genetic analyses of 16 populations (**Chapter**

II) provided evidence for wide prevalence of hybridization between *Ch. montanus* and *Ch. parallelus* in the wild. As no complete admixture was found in the examined population, it is assumed that hybridization only occurs in the ecotones between wetlands and drier parts. Reproductive barriers such as habitat isolation, behavior or phenology seem to prevent the genetic swamping of *Ch. montanus* populations. Although a behavioral experiment showed that mate choice presents an important reproductive barrier between both species, the experiment also revealed that reproductive barriers could be altered by environmental change, because interbreeding increased with increasing heterospecific frequency.

Chapter III analyzes the impact of extreme climatic events on population dynamics and interspecific hybridization. A mark-recapture analysis combined with weather records over five years provides evidence that the embryonic development in *Ch. montanus* is vulnerable to extreme climatic events, such as droughts during the egg stage. Strong population declines in *Ch. montanus* lead to a disequilibrium between *Ch. montanus* and *Ch. parallelus* populations and increases the risk of hybridization. The highest hybridization risk was found in the first weeks of a season, when both species had an overlapping phenology. Furthermore, hybrids were generally localized at the edge of the *Ch. montanus* distribution with higher heterospecific encounter probabilities. The hybridization rate reached up to 19.6%. The genetic analyses in Chapter II and III show that hybridization differentially affects specialists and generalists. While generalists may benefit from hybridization by an increasing genetic diversity, such a positive correlation was not found for *Ch. montanus*. The results underline the importance of reproductive barriers for the co-existence of these sympatric species. However, climate change and other anthropogenic disturbances alter reproductive barriers and promote hybridization, which may threaten small populations by genetic displacement.

As anthropogenic hybridization is recognized as a major threat to biodiversity, it should be considered in environmental law and policy. In **Chapter IV** the role of hybrids and hybridization in three levels of law (international, European, national) and the historical backgrounds of hybrids becoming a part of legal instruments is analyzed. Due to legal uncertainties and the complexity of this topic a legal assessment of hybrids is challenging and argues for species-specific approaches. Nonetheless, existing legal norms provide a suitable basis, but need to be specified. Finally, this chapter discusses different opportunities for the management of hybrids and hybridization in a conservation perspective and their necessity.

Zusammenfassung

Klimawandel und Lebensraumfragmentierung verändern natürliche Lebensräume vieler Feuchtgebietsarten und führen zu neuen Artenzusammensetzungen in diesen Ökosystemen. Während die direkten Auswirkungen des Klimas oft bekannt sind, sind die indirekten Auswirkungen auf biotische Interaktionen weitgehend unerforscht. Vor allem die Konsequenzen für wenig mobile Arten, deren Lebensraum ohnehin schon durch Fragmentierung stark eingeschränkt ist, bedürfen mehr Aufmerksamkeit im Naturschutz. In der vorliegenden Arbeit wurde der Sumpfgrashüpfer, *Chorthippus montanus,* als Modellart für die Analyse der Auswirkungen des Klimawandels auf die Hybridisierung mit einer weit verbreiteten Schwesterart gewählt. *Ch. montanus* ist ein Habitatspezialist, der aufgrund seiner Ansprüche während der Embryonalentwicklung auf Feuchtgebiete angewiesen ist. In weiten Teilen der Paläarktis kommt er sympatrisch mit seiner weitverbreiteten Schwesterart *Ch. parallelus* vor. Die nahe Verwandtschaft und ähnlichen Balzgesänge lassen vermuten, dass beide Arten in syntopen Populationen hybridisieren. Da beide Arten flugunfähig und die Populationen von *Ch. montanus* oft stark fragmentiert sind, könnte eine durch Klimawandel bedingte Abnahme der Habitatqualität das Hybridisierungsrisiko erhöhen und somit letztendlich zur genetischen Verdrängung dieser gefährdeten Art führen. In der vorliegenden Arbeit wurde mithilfe eines multiplen Methodenansatzes (Laborexperimente, Populationsgenetik, Fang-Wiederfang-Studie) der Einfluss von Umweltfaktoren (insbesondere des Klimas) auf den Lebenszyklus, die Populationsdynamik und interspezifische Interaktionen des Sumpfgrashüpfers untersucht. Des Weiteren wird die Rolle von Hybridisierung und Hybriden im Umweltrecht diskutiert und bewertet.

In **Kapitel 1** wurde mithilfe von Klimakammer-Experimenten der Einfluss von Umweltfaktoren (Temperatur, Dichte) auf den Lebenszyklus des Sumpfgrashüpfers untersucht. Aufgrund der Immobilität von *Ch. montanus* sollte das Experiment zunächst die Hypothese testen, dass hohe Temperaturen oder Dichten die Entwicklung makropterer Morphen begünstigen und somit die Ausbreitung in andere Lebensräume ermöglichen. Obwohl diese Hypothese nicht bestätigt werden konnte, zeigten sich deutliche Auswirkungen auf den Lebenszyklus. Wie bereits bei anderen Orthopteren beobachtet, folgte *Ch. montanus* der umgekehrten „Temperatur-Größen-Regel“, d.h. die Körpergröße stieg mit zunehmender Temperatur. Zusätzlich zeigte sich in Übereinstimmung mit der Nischendimorphismus-Hypothese, dass beide Geschlechter unterschiedlich auf Konkurrenz bei niedriger Temperatur reagierten. Die Nischendimorphismus-Hypothese besagt, dass sexuelle Dimorphismen auf den unterschiedlichen reproduktiven Rollen der Geschlechter beruhen. Tatsächlich erreichten

die Männchen der Art unter Konkurrenz geringere Körpergrößen, während die Weibchen eine verlängerte Entwicklungszeit aufwiesen. Dies ist der erste Nachweis für eine geschlechtsspezifische phänotypische Plastizität bei dieser Art. Diese könnte sich als nachteilig für *Ch. montanus* herausstellen, da eine höhere Dichte eine innerartliche Phänologie-Verschiebung zur Folge hätte, die den Reproduktionserfolg dieser Art reduzieren und das Hybridisierungsrisiko mit der Schwesterart erhöhen könnte.

Im **Kapitel II** und **III** wurden interspezifische Interaktionen zwischen den beiden Schwesterarten *Ch. montanus* und *Ch. parallelus* untersucht. Dabei liefert die populationsgenetische Analyse in **Kapitel II** den ersten Nachweis über die Hybridisierung in freilebenden Populationen der beiden Arten. Reproduktive Barrieren (Habitat, Verhalten, Phänologie) scheinen dabei eine komplette genetische Durchmischung zu verhindern. Ein Verhaltens-Experiment bestätigte, dass Partnerwahl eine wichtige Rolle als reproduktive Barriere einnimmt, diese aber durch Umweltfaktoren verändert werden kann. So steigt das Hybridisierungsrisiko mit ansteigender heterospezifischer Dichte. Dies bestätigen auch die populationsökologischen und -genetischen Analysen in **Kapitel III**. Des Weiteren zeigten die genetischen Analysen, dass Hybridisierung unterschiedliche Auswirkungen auf Spezialisten und Generalisten hat. Während *Ch. parallelus* von Hybridisierung durch einen Anstieg der genetischen Diversität profitiert, konnte bei *Ch. montanus* keine positive Auswirkung festgestellt werden. Klimawandel und extreme Wetterereignisse erhöhen das Hybridisierungsrisiko drastisch, indem sie zu starken Populationseinbrüchen, einer Phänologie-Verschiebung oder einer Verschmelzung der Habitatgrenzen führen. Besonders Spezialisten wie *Ch. montanus* zeigen eine hohe Empfindlichkeit gegenüber extremen Wetterereignissen. So zeigten die Analysen, dass Populationseinbrüche vor allem auf die langen Trockenperioden während der Embryonalentwicklung zurückzuführen sind.

Anthropogene Hybridisierung stellt eine Gefahr für Biodiversität dar und sollte daher auch im Hinblick auf Umweltrecht und Umweltpolitik näher analysiert werden. In **Kapitel IV** wird der historische Hintergrund von Hybriden im Naturschutzrecht dargestellt und die Rolle von Hybridisierung in den drei Rechtsgebieten internationales, Europäisches und nationales Umweltrecht bewertet. Aufgrund von Rechtsunsicherheiten und der Komplexität dieses Themas, stellt die rechtliche Bewertung von Hybriden und Hybridisierung eine Herausforderung dar. Obwohl die Analyse generell eher für artspezifische Lösungen spricht, sollte Hybridisierung einen eigenständigen Status im Naturschutzrecht erhalten. Bereits existierende Rechtsnormen oder Ansätze liefern dabei eine gute Grundlage, die im Einzelnen aber konkretisiert werden müssen.

Zusammenfassung - 1 Seite

Klimawandel und Lebensraumfragmentierung verändern natürliche Lebensräume vieler Feuchtgebietsarten und führen zu neuen Artenzusammensetzungen in diesen Ökosystemen. Während die direkten Auswirkungen des Klimas oft bekannt sind, sind die indirekten Auswirkungen auf biotische Interaktionen weitgehend unerforscht. In der vorliegenden Arbeit wurde der Sumpfgrashüpfer, *Chorthippus montanus*, als Modellart gewählt, um die Auswirkungen von Klimaänderungen auf seinen Lebenszyklus, die Populationsdynamik und Hybridisierung mit seiner weit verbreiteten Schwesterart *Ch. parallelus* zu untersuchen. *Ch. montanus* ist ein Habitatspezialist, der aufgrund seiner Ansprüche während der Embryonalentwicklung auf Feuchtgebiete angewiesen ist. Die nahe Verwandtschaft und ähnliche Balzgesänge beider Arten haben bereits in der Vergangenheit vermuten lassen, dass sie in syntopen Populationen hybridisieren. Dies konnte in dieser Arbeit mit Hilfe molekulargenetischer Methoden bestätigt werden. Des Weiteren zeigte sich, dass Habitatgeneralisten von Hybridisierung profitieren können, da hierdurch ihre genetische Diversität steigt. Bei Spezialisten wie *Ch. montanus* dagegen sinkt die genetische Diversität und sie scheinen ihre genetische Integrität zu verlieren. Eine Fang-Wiederfang-Studie über fünf Jahre bestätigte, dass vor allem eine Kombination aus drei reproduktiven Barrieren (Phänologie, Habitat, Partnerwahl) die genetische Verdrängung des Sumpfgrashüpfers in den untersuchten Populationen verhindert hat. Diese Barrieren können allerdings durch extreme Wetterereignisse verändert oder sogar aufgelöst werden, indem sie zu starken Populationseinbrüchen und einem Ungleichgewicht der syntopen Populationen führen. Ein weiteres Experiment zeigte, dass Umweltfaktoren auch den Lebenszyklus der Art beeinflussen. In Übereinstimmung mit der Nischendimorphismus-Hypothese konnte der erste Nachweis einer geschlechtsspezifischen phänotypischen Plastizität für diese Art erbracht werden. Diese könnte sich allerdings als nachteilig für *Ch. montanus* herausstellen, da eine höhere Dichte eine innerartliche Phänologie-Verschiebung bewirken kann, die den Reproduktionserfolg dieser Art reduzieren und das Hybridisierungsriko mit der Schwesterart erhöhen könnte. Besonders für seltene Arten mit kleinen Populationen stellt Hybridisierung eine Gefahr dar. Das letzte Kapitel der vorliegenden Arbeit beschäftigt sich daher mit der Rolle von Hybriden und Hybridisierung im Umweltrecht. Die rechtliche Bewertung stellt aufgrund von Rechtsunsicherheiten und der Komplexität des Themas eine Herausforderung dar. Obwohl die Analyse generell eher für artspezifische Lösungen spricht, sollte Hybridisierung einen eigenständigen Status im Naturschutzrecht erhalten. Bereits existierende Rechtsnormen oder Ansätze liefern dabei eine gute Grundlage, die im Einzelnen aber konkretisiert werden müssen.

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09.2010	Diplomarbeit im Institut für Sinnesökologie, Fachbereich Biologie, Universität Düsseldorf, Arbeitsgruppe Prof. Dr. Klaus Lunau *Titel*: „Angeborene und erlernte Farbpräferenzen bei Honigbienen und Hummeln“
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2007-2011	Mitarbeiterin im Zoo Duisburg (Zoobegleiterin, Planung und Durchführung von Artenschutzkampagnen)

Organisation von Workshops

Mitorganisation des DFG-Workshops “Anthropogene Störungen mariner Ökosysteme in Deutschland - eine natur- und rechtswissenschaftliche Bewertung” (2012)

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Erklärung

Hiermit erkläre ich, dass mir die derzeitige Promotionsordnung bekannt ist und ich die vorliegende Dissertation selbständig verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ergebnisse anderer Beteiligter sowie die inhaltlich und wörtlich aus anderen Werken entnommenen Stellen und Zitate sind als solche kenntlich gemacht. Die vorliegende Dissertation hat in ähnlicher oder gleicher Form noch keiner anderen Prüfungsbehörde vorgelegen oder wurde von dieser als Teil einer Prüfungsleistung angenommen.

Ort/Datum

Katja Rohde

„In der Natur ist alles mit allem verbunden, alles durchkreuzt sich, alles wechselt mit allem, alles verändert sich eines in das andere.“

- Gotthold Ephraim Lessing -