The role of hybridization in the colonization of newly opened habitats.

Johanna Griebel

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Erstgutachter: Prof. Dr. Justyna Wolinska

Zweitgutachter: Prof. Dr. Herwig Stibor

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ABSTRACT

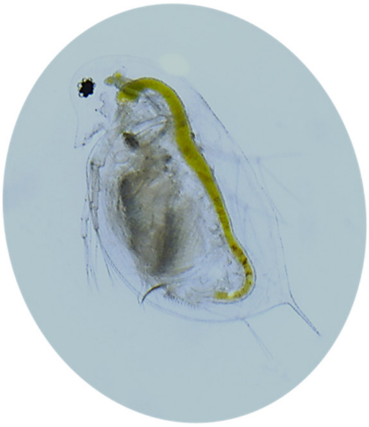
For a long time the role of hybridization in ecological and evolutionary process has been underestimated in the animal kingdom. In contrast, the number of studies reporting successful hybridization has increased tenfold in the last decades. It has often been reported that the establishment of hybrids has been connected with their abilities to colonize new and extreme environments. The aim of this thesis was to evaluate which processes lead to frequently observed hybrid establishment in the *Daphnia longispina* species complex. *Daphnia* are cyclical parthenogens with an asexual and a sexual phase of reproduction, during which hybrids are produced. Hybrids of this species complex are known to be less successful in sexual reproduction compared to their parental species. As *Daphnia* mainly survive winter by producing sexual resting eggs, the main focus was set on how hybrids survive winter and reach high abundances in spring. Therefore, *Daphnia* individuals were sampled during spring over six consecutive years and monthly during one year in ten shallow lakes strongly affected by winter. Using microsatellite data the following three scenarios were tested: 1) recurrent hybridization events, 2) survival as asexual females during winter and 3) establishment of hybrids via migrants. Furthermore, several experimental surveys were performed to find possible traits that facilitate the dominance of a “successfully hybrid” clone, as observed in one of the tested lakes in two consecutive years. A mathematical model was created to evaluate various possible traits that might facilitate the establishment of hybrids. In the spring samples high levels of linkage disequilibrium and observed heterozygosity were found in hybrids, indicating that they mainly survive as asexual females during winter. Enhanced survival of asexual females in hybrids was confirmed in the experiment exposing hybrids and parental species to winter conditions. In addition, the mathematical model confirmed that an enhanced overwintering as asexual females can lead to an establishment of hybrids. Interestingly, field data showed that cold winters resulted in lower numbers of hybrid genotypes in the following spring suggesting a selection of hybrids with high capabilities to survive winter. In the mathematical model hybrids dominated the community during cold winters, while warm winters could lead to the loss of such an advantage. Low migration rates and high genetic differentiation in hybrids compared to the parental species suggest that migration of hybrids could be excluded. Overall the results of my thesis suggest that an enhanced parthenogenetic reproduction of *Daphnia* hybrids during winter can facilitate their successful establishment. Furthermore, such an advantage depends on the severity of the winter.

ZUSAMMENFASSUNG

Lange Zeit wurde die Rolle der Hybridisierung in ökologischen und evolutionären Prozessen im Reich der Tiere unterschätzt. Im Gegensatz dazu stieg die Anzahl der Studien, die über erfolgreiche Hybridisierung berichten, in den letzten Jahrzehnten um das Zehnfache. Diese Studien berichten häufig darüber, dass die Etablierung von Hybriden mit ihrer Fähigkeit zusammenhängt, neue und extreme Umwelten zu kolonisieren. Das Ziel dieser Arbeit besteht darin zu evaluieren, welche Prozesse zu der häufig beobachteten Etablierung von Hybriden im *D. longispina* Artenkomplex führen. *Daphnien* sind zyklische parthenogenetische Organismen mit einer asexuellen und einer sexuellen Reproduktionsphase, während derer Hybride gebildet werden. Hybride dieses Artenkomplexes sind dafür bekannt, dass sie im Vergleich zu ihren Elternarten weniger erfolgreich in der sexuellen Reproduktion sind. Da *Daphnien* den Winter vor allem durch die Produktion sexueller Dauereier überleben, wurde ein Hauptaugenmerk darauf gerichtet, wie Hybride den Winter überleben und hohe Häufigkeiten im Frühling erreichen. Dazu wurden *Daphnien*-Individuen über einen Zeitraum von sechs aufeinander folgenden Jahren sowie monatlich innerhalb eines Jahres in zehn seichten Seen, welche stark durch den Winter beeinflusst sind, beprobt. Mithilfe von Mikrosatellitendaten wurden drei Szenarien getestet: 1) wiederkehrendes Vorkommen von Hybridisierung, 2) das Überleben von asexuellen Weibchen während des Winters und 3) die Etablierung von Hybriden durch Migranten. Des Weiteren wurden mehrere experimentelle Studien durchgeführt, um mögliche Merkmale zu finden, die die Dominanz eines „erfolgreichen Hybrid-Klons“, der in einem der beprobten Seen in zwei aufeinander folgenden Jahren beobachtet wurde, erklären. Es wurde außerdem ein mathematisches Modell entwickelt, um verschiedene mögliche Merkmale zu evaluieren, die eine erfolgreiche Etablierung von Hybriden ermöglichen. In den Proben, die im Frühling entnommen wurden, wurden hohe Werte von *Linkage Disequilibrium* und beobachteter Heterozygotie gefunden, die auf ein hauptsächliches Überleben von asexuellen Weibchen über den Winter hinaus hinweisen. Ein verbessertes Überleben von asexuellen Weibchen in Hybriden wurde in einem Experiment bestätigt, in dem Hybride und Elternarten Winterbedingungen ausgesetzt waren. Außerdem bestätigte das mathematische Modell, dass ein verbessertes Überleben durch asexuelle Weibchen zu einer Etablierung von Hybriden führen kann. Interessanterweise zeigten Felddaten, dass kalte Winter zu einer geringeren Anzahl verschiedener Hybridgenotypen im folgenden

Frühling führen können. Das weist darauf hin, dass Hybride mit einer hohen Fähigkeit den Winter zu überleben, selektiert werden. In dem mathematischen Modell dominierten Hybride die Gemeinschaft in kalten Wintern, während warme Winter zu einem Verlust dieses Vorteils führten. Niedrige Migrationsraten und eine hohe genetische Differenzierung in Hybriden im Vergleich zu den Elternarten deuten an, dass Migration von Hybriden ausgeschlossen werden kann. Insgesamt zeigen die Ergebnisse der Arbeit, dass eine verbesserte parthenogenetische Reproduktion von *Daphnien*-Hybriden während des Winters eine erfolgreiche Etablierung von Hybriden ermöglicht. Dabei hängt dieser Vorteil von der Stärke des Winters ab.

CHAPTER 1: INTRODUCTION



Hybridization

Hybridization in animals

Hybridization is defined as the interbreeding of individuals from two populations or groups of populations, which are distinguishable on the basis of one or more heritable characters (Dowling and Secor 1997). In many cases post- and prezygotic barriers prevent successful hybridization between such populations (summarized in Coyne and Orr 2004). However, these barriers are not always complete. In the plant kingdom the role of hybridization in evolutionary and ecological processes has been acknowledged for a long time (Grant 1981; Rieseberg and Brunsfeld 1992; Stebbins 1959). In contrast, in the animal kingdom it has been considered of less importance: "The total weight of evidence contradicts the assumption that hybridization plays a major role among higher animals…" (Mayr 1963, p. 133). First, the crossing between divergent lineages were regarded to result mainly in offspring with a reduced viability and/or fertility (Mayr 1963). The reason for the reduced fitness of hybrid offspring was seen in the complexity of organs and tissues in animals, that requires a large amount of genes interacting with each other (Dobzhansky 1951). Genes of the parental species have been under selection pressure for a long time and they might be co-adapted within the parental species. Sexual recombination during the mating of divergent populations are likely to break up such interacting genes (Mayr 1963). Second, if reproductive isolation would be weak enough to facilitate successful hybrids, species that coexist would not be maintained, if they are not geographically isolated (Mayr 1963). Therefore, introgression of genes via hybridization would be unlikely (Mayr 1963).

In the last decades, hybridization in the animal kingdom has gained more attention (reviewed in Schwenk et al. 2008) and hybrids of various groups were found to show the same or even higher fitness than the parental species. For example in the Darwin finches from the Galapagos Island, hybrids between *Geospiza fortis* and *G. scandens* were favored during a dry period caused by a rare El Nino event (Grant and Grant 1996). Due to the drought, seeds became smaller and could be eaten easier by hybrids having a smaller beak than the parental species. In the North American leopard frog, hybrids between *Rana blairi* and *R. sphenocephala* had the highest proportion of individuals that survived and metamorphosed compared to their parental species in three different aquatic habitats (Parris 2001). These observations contradict the traditional view of hybrids always being less fit than their parental species. It is assumed that the combination of isolated genomes results in a distribution of differently fit phenotypes of hybrids. Such phenotypic traits can be outside of the distribution of the parental species, having negative but also positive effects on the fitness of hybrids (Abbott et al. 2013). Positive effects could be the result of heterosis, the novel combination of genes or the combination of advantageous genes (Burke and Arnold 2001). Even if most hybrid genotypes would be less fit than the parental species, natural selection can pick out fit genotypes (Barton 2001). Selection on hybrids can be endogenous or exogenous (Burke and Arnold 2001). Endogenous selection means that the fitness of hybrids is independent of the environment, such as incompatibilities of genes (Burke and Arnold 2001). In the *tension zone model* (Barton and Hewitt 1985) it is thought that the success of hybrids depends rather on the balance between the reduced fitness and their dispersal abilities. Exogenous selection means that the fitness of hybrids is dependent on environmental factors (Burke and Arnold 2001). The *gradient ecotone model* (Endler 1977) describes how environmental gradients result in gradients of selection pressure. While the parental species perform best at the extreme ends of the gradients, hybrids are intermediate. According to the *bounded hybrid superiority model* (Moore 1977; Moore and Koenig 1986) parents are adapted to different environments, while hybrids are adapted to neither of them, but new ones. Such environments are often considered to be extreme compared to the original environments of the parental species (Seehausen 2004). For example, hybrids of the sunflowers are well adapted to sand dunes, desert floors and salt marshes; habitats where the parental species cannot establish (Rieseberg et al. 2006). In spadefoot toads hybrids have longer development times, which makes them less fit in the parental environments, but are beneficial during long dry periods (Pfennig 2007).

Additionally, hybridization could allow for a fast introduction of variation. Within one generation more combinations of genes could be created than by selection on neutral alleles with mutations (Schwenk and Spaak 1997). Also groups of genes that are stabilized could be transferred fast between populations (Dowling and Secor 1997). Moreover, hybridization introduces genetic variation that can reduce inbreeding depression during periods of small population size (Dowling and Secor 1997). In changing environments rapid emerging variation could be advantageous, particularly when new ecological niches arise (Dowling and Secor 1997). Indeed, it has been observed that hybrids usually occupy environments which are different from those of their parental species (reviewed in Seehausen 2004). Hybridization therefore can be a factor driving adaptive radiation (Seehausen 2004) as observed in the *Heliconius* butterflies. *Heliconus* butterflies divide in a variety of species. These species have a large variation in wing patterns, which is unlikely to be the result of mutation, but to have their origin in several hybridization events (Gilbert 2003).

Parthenogenesis and hybridization

Parthenogenesis is the development of embryos without fertilization. The offspring can be genetically identical with the mother (clones). For a long time, clonal reproduction has been seen as an evolutionary dead end, because deleterious mutations would become fixed and variability reduced (Delmotte et al. 2003). Moreover, genetic drift would lead to a decrease in variability and finally to the extinction of parthenogenetic reproduction. Therefore, clonal reproduction would decrease the rate of adaptive evolution, rate of speciation and increase the rate of extinction (Vrijenhoek 1979). In contrast, in some animal species clonal parthenogenetic lineages established and showed to have higher variability than expected (for examples see Kearney 2005). Different theories have been developed to explain the contradiction between expectation and observation. In the Frozen Niche Variation (FNV) theory, favored gene complexes in clonal lineages are not broken up via sexual reproduction (Vrijenhoek 1979). Variation is frozen within clonal lineages. Often such favored traits are associated with special niches at the edges of the environment of the sexual parental species, and clones can disperse to environments with similar settings (Kearney 2005). In the FNV theory asexual individuals have to arise constantly from sexual populations, leading to high genetic diversity (Vrijenhoek 1979; Vrijenhoek and Parker 2009). In the frog *Rana esculanta*, different clones have different fitness peaks for specific habitats, expressing a superiority as clonal mixture rather as single clones (Semlitsch et al. 1997). Alternatively, certain clones can show an adaptation to a wide range of environments and intermediate success in life history traits, facilitating a broad establishment and dispersal of these general purpose genotypes (GPGs) (Vrijenhoek 1979; Vrijenhoek and Parker 2009). In such a case, mutations are the only source of variation leading to a low genetic diversity. An example of an GPG is the asexual ostracod *Darwinula stevensoni,* that shows a broad tolerance for salinity and temperature and has a broad geographical and ecological distribution which can explain its long-term persistence (Van Doninck et al. 2002). In addition, parthenogenesis can have some short-term advantages, for example the ability of colonization with one female only and the amplification of co-adapted gene combinations (Delmotte et al. 2003).

Parthenogenetic lineages are often associated with specific environmental settings (geographical parthenogenesis, reviewed in Kearney 2005). Such environments are extreme habitats such as high altitudes and latitudes, deserts islands, disturbed and ecotone habitats. It has been proposed that geographic parthenogenesis is likely to be a pattern of hybridization and the advantage of hybrids in newly opened environments (Kearney 2005). Parthenogenesis often occurs in populations that originated via hybridization (Delmotte et al. 2003). During hybridization co-adapted gene complexes responsible for the meiotic cell division can be broken up inducing parthenogenetic reproduction. Interestingly, almost all vertebrates that reproduce parthenogenetically have hybrid origin (Avise et al. 1992). Hybrids might successfully establish also because of their ability of parthenogenetic reproduction (e.g. aphids, Delmotte et al. 2003; freshwater snails, Johnson 2005; edible frogs, Semlitsch 1993; waterfleas, Spaak and Hoekstra 1995), which compensates for otherwise reduced hybrid abilities to reproduce sexually (Schwenk and Spaak 1997).

Daphnia as a model system for hybridization

More than 100 *Daphnia* species are found around the world in most standing freshwater, from large lakes to small pools and temporary rockpools (Adamowicz et al. 2009; Petrusek et al. 2012). *Daphnia* is a key species in the food webs of lakes and ponds. They are grazers on algae and serve as food for planktivorous fish and carnivorous invertebrates (Lampert 2011). *Daphnia* are cyclical parthenogens with an asexual and a sexual phase. During the asexual phase females produce genetically identical offspring. During the sexual phase females produce genetically identical diploid males or haploid resting eggs (Hebert 1978). These resting eggs are protected by an ephippium, which makes them resistant to freezing and desiccation. Therefore, the phase of sexual reproduction is mostly induced during harsh conditions. The production of males and sexual females is dependent on temperature, food quantity and quality, density and the photoperiod (Hebert 1978; Innes 1997; Spaak 1995). The induction of resting egg production is more species-specific than the production of males (Spaak 1995). After fertilization resting eggs are released into the water column, where they float or sink to the sediment of the lake. Lake sediments often contain large numbers of *Daphnia* diapausing eggs that have been accumulated over dozens of years (10^3-10^5 eggs per square meter, Brendonck and De Meester 2003). Resting eggs also provide the main dispersal stage of *Daphnia*, as they can be transported via wind or birds (Bilton et al. 2001; Figuerola et al. 2005).

In the temperate zones *Daphnia* densities vary strongly throughout the year. Early in the year numbers of *Daphnia* increase rapidly with exponential growth (Ebert 2005). Later on, growth is reduced by density-dependent competition, which can be followed by a midsummer decline, due to overgrazing on the food resources of *Daphnia* such as phytoplankton (Sommer et al. 1986). During cold periods (i.e. winter) *Daphnia* occur at low densities or disappear completely. *Daphnia* can overwinter by persisting as asexual lineage in the water column or via sexually produced resting eggs, which hatch under favorable conditions (Hamrová et al. 2011; Lampert et al. 2010). Surviving as asexual female can lead to a competitive advantage in spring, when they can quickly reproduce parthenogentically, and their offspring are born during an algae bloom (Sommer et al. 1986). The early occurrence of such surviving females may then lead to dominance later on (i.e. priority effects, Louette and De Meester 2007). However, survival as asexual females can be risky if lakes get anoxic during ice cover or freeze to the bottom of the lake (Lampert et al. 2010).

Hybridization in *Daphnia*

In the water flea *Daphnia* hybrids are common worldwide and have been documented in several species complexes (Colbourne et al. 1998; Hebert 1985; Keller et al. 2008). The *Daphnia longispina* species complex is the most commonly found complex in Europe (mainly consisting of *D. galeata, D. longispina, D. cucullata* and their interspecific crosses; taxonomy revised in Petrusek et al. 2008). Hybrids of this complex are less successful in sexual reproduction compared to the parental species (Keller and Spaak 2004; Keller et al. 2007; Spaak et al. 2004). This is because hybrids produce less resting eggs and resting eggs containing hybrids have lower hatching success. Moreover, members of this species complex do not reproduce at random, resulting in lower numbers of resting eggs containing hybrids. In contrast, during the parthenogenetic life cycle hybrids of this complex can show the same or even higher fitness than the parental species. Such fitness advantages occur during certain environmental conditions for example when fed with different quantities (e.g. Boersma and Vijverberg 1994) or qualities of food (e.g. Seidendorf et al. 2007), exposed to different temperatures (e.g. Weider and Wolf 1991), predation (e.g. Declerck and Meester 2003), or parasite pressure (e.g. Wolinska et al. 2006). For example, *D. galeata* × *D. longispina* hybrids performed worse at 20 °C compared to their parental species but showed higher fitness values when raised at 14 °C (Weider and Wolf 1991). Environmental changes in time and space may therefore provide open windows for the successful establishment of hybrids in the *Daphnia longispina* complex (Spaak and Hoekstra 1995). In fact, field observations indicate that *Daphnia* hybrids may become more abundant or outcompete their parental species during certain time periods (Hebert and Finston 2001; Keller et al. 2008; Yin et al. 2012).

Aim of the thesis

In this thesis, I examined hybridization in the *D. longispina* complex and its role in the establishment of newly opened habitats. To evaluate hybridization across space and time I collected genetic data from the hybridizing complex across habitats and generation. I screened *Daphnia* communities in ten artificial lakes (flooded gravel pits) in and around Munich (Fig. 1). These lakes have been created recently (max. age 80 years), resulting in newly founded *Daphnia* communities. In addition, these lakes are rather shallow with a maximum depth of 16 m, which makes them strongly affected by temperature decline in winter. During winter *Daphnia* abundances fall under detection level in these lakes, leading to the opening of the habitats in spring. The maximum distance between lakes is 30 km. Altogether, these features make these lakes an ideal system to study hybrid establishment in newly opened habitats on a small geographical scale.

Figure 1: Ten lakes sampled in and around Munich.

The aim of the thesis was to evaluate how *Daphnia* hybrids establish in spring by testing three contrasting scenarios: 1) recurrent hybridization events, 2) survival as asexual females during winter and 3) establishment of hybrids via migrants. Moreover, using different experimental set-ups (carrying capacity, temperature and crowding) a "successful hybrid" clone (i.e. the clone that dominated an entire *Daphnia* community in two consecutive years) was compared with other clones of the parental species and their hybrids. Finally, observed parameters that might facilitate the establishment of hybrids were analyzed in a mathematical model.

My PhD thesis contains the following chapters:

Chapter 2: *Daphnia* communities of ten lakes were sampled every spring during six consecutive years. Individuals were screened at 15 microsatellite loci to identify *Daphnia* species and their hybrids. The establishment of hybrids in the beginning of spring was examined by analysing the clonal and genetic diversity of hybrids, migration, temporal and spatial differentiation as well as the correlation with the severity of the winter in comparison to the parental species.

Chapter 3: *Daphnia* communities were sampled every month during one growing season (April-November). Individuals were screened at 15 microsatellite loci to identify *Daphnia* species and their hybrids. Furthermore, environmental characteristics of the lakes (e.g. phosphorus or nitrate level) were tested for correlation with the abundance of parental species and hybrids. Clonal diversity was followed over the year to examine if clonal diversity will decrease over the season (clonal erosion). Furthermore, we tested if hybrids are more likely to be produced locally or dispersed from other lakes.

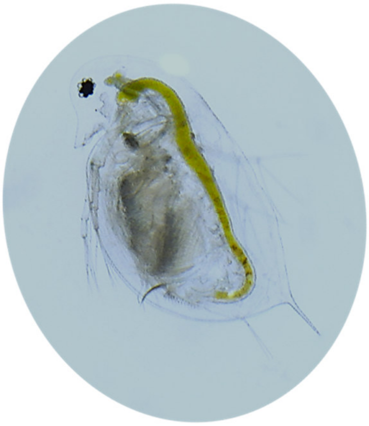
Chapter 4: In Chapter 2, we found one hybrid genotype from the lake Feldmochinger See to dominate the *Daphnia* community, in two consecutive years. To examine traits which facilitate the superiority of these "successful hybrid", I conducted a competition experiment and life history experiments under different conditions (temperature, crowding, carrying capacity and simulated winter). Moreover, abundances of the "successful hybrid" were tested for correlations with the strength of the winter.

Chapter 5: Here we employed a mathematical model to examine conditions that could explain the frequently observed establishment of hybrids in the *Daphnia longispina* complex. Therefore, we simulated the co-occurrence of parental species and hybrids over several years. Specifically, we compared the outcome of hybrid and parental species competition under different scenarios such as including a reduction of sexual reproduction in hybrids and simulating their enhanced survival over winter as asexual females.

CHAPTER 2: PARENTAL AND HYBRID *DAPHNIA* FROM THE *D. LONGISPINA* COMPLEX: LONG-TERM DYNAMICS IN GENETIC STRUCTURE AND SIGNIFICANCE OF OVERWINTERING STRUCTURES

Johanna Griebel, Sabine Gießler, Mingbo Yin, Justyna Wolinska (2016)

Journal of Evolutionary Biology



Abstract

In recent decades, hybridization has become a focus of attention because of its role in evolutionary processes. However, little is known about changes in genetic structure within and between parental species and hybrids over time. Here, we studied processes of genetic change in parental species and hybrids from the Daphnia longispina complex (Crustacea, Cladocera) over a period of six years across ten habitats. These cyclical parthenogens respond to fluctuating environments by switching from asexual to sexual reproduction. Importantly, sexually produced diapausing eggs, which resist extreme conditions such as low temperatures and serve as dispersal stages, are produced to a lower extent by hybrids. Long-term microsatellite data revealed clear differences between hybrids and parental species. In hybrids, clonal diversity values were lower, whereas heterozygosity and linkage disequilibrium values were higher compared to parental species. Clonal diversity of hybrids responded to the strength of the winter, with cold winters resulting in few genotypes in the following spring. In time windows when only asexual hybrid females survive, priority effects will favour the establishment of the hybrid offspring before hatchlings from parental diapause eggs can enter the community. The constant high levels of heterozygosity maintained by clonal reproduction in hybrids might lead to their successful establishment over time, when they are able to escape competition from both parental species. Although we found evidence that hybrids diversity depends on fluctuating environments, a direct link between hybrid abundance and the strength of winter was missing. Because of reduced adaptability in clonally reproducing hybrids, multiple factors must contribute to promoting their long-term success in fluctuating environments.

Full-text article:

http://onlinelibrary.wiley.com/doi/10.1111/jeb.12828/abstract

CHAPTER 3: HYBRIDIZING *DAPHNIA* COMMUNITIES FROM TEN NEIGHBOURING LAKES: SPATIO-TEMPORAL DYNAMICS, LOCAL PROCESSES, GENE FLOW AND INVASIVENESS

Mingbo Yin, Sabine Gießler, Johanna Griebel, Justyna Wolinska (2014)

BMC Evolutionary Biology, 14: 80

Abstract

Background

In natural communities of cyclical parthenogens, rapid response to environmental change is enabled by switching between two reproduction modes. While long periods of asexual reproduction allow some clones to outcompete others, and may result in "clonal erosion", sexual reproduction restores genetic variation in such systems. Moreover, sexual reproduction may result in the formation of interspecific hybrids. These hybrids can then reach high abundances, through asexual clonal reproduction. In the present study, we explored genetic variation in water fleas of the genus Daphnia. The focus was on the short-term dynamics within several clonal assemblages from the hybridizing Daphnia longispina complex and the impact of gene flow at small spatial scales.

Results

Daphnia individuals belonged either to the parental species D. galeata and D. longispina, or to different hybrid classes, as identified by 15 microsatellite markers. The distribution and genotypic structure of parental species, but not hybrids, corresponded well with the geographical positions of the lakes. Within parental species, the genetic distance among populations of D. galeata was lower than among populations of D. longispina. Moreover, D. galeata dominance was associated with higher phosphorous load. Finally, there was no evidence for clonal erosion.

Conclusions

Our results suggest that the contemporary structure of hybridizing Daphnia communities from ten nearby lakes is influenced by colonization events from neighbouring habitats as well as by environmental factors. Unlike the parental species, however, there was little evidence for successful dispersal of hybrids, which seem to be produced locally. Finally, in contrast to temporary Daphnia populations, in which a decrease in clonal diversity was sometimes detectable over a single growing season, the high clonal diversity and lack of clonal erosion observed here might result from repeated hatching of sexually produced offspring. Overall, our study provides insights into spatio-temporal dynamics in a hybridizing Daphnia species complex in a recently established lake system, and relates genetic similarities of populations to a scenario of secondary invasion enhanced by environmental factors.

Full-text article:

http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-14-80

CHAPTER 4: EXTREME ENVIRONMENTS FACILITATE HYBRID SUPERIORITY – THE STORY OF A SUCCESSFUL *DAPHNIA GALEATA* × *LONGISPINA* HYBRID CLONE

Johanna Griebel, Sabine Gießler, Monika Poxleitner, Amanda Navas Faria, Mingbo Yin, Justyna Wolinska (2015)

Plos ONE, 10(10)

Abstract

Hybridization within the animal kingdom has long been underestimated. Hybrids have often been considered less fit than their parental species. In the present study, we observed that the *Daphnia* community of a small lake was dominated by a single *D. galeata* × *D. longispina* hybrid clone, during two consecutive years. Notably, in artificial community set-ups consisting of several clones representing parental species and other hybrids, this hybrid clone took over within about ten generations. Neither the fitness assay conducted under different temperatures, or under crowded and non-crowded environments, nor the carrying capacity test revealed any outstanding life history parameters of this hybrid clone. However, under simulated winter conditions (i.e. low temperature, food and light), the hybrid clone eventually showed a higher survival probability and higher fecundity compared to parental species. Hybrid superiority in cold-adapted traits leading to an advantage of overwintering as parthenogenetic lineages might consequently explain the establishment of successful hybrids in natural communities of the *D. longispina* complex. In extreme cases, like the one reported here, a superior hybrid genotype might be the only clone alive after cold winters. Overall, superiority traits, such as enhanced overwintering here, might explain hybrid dominance in nature, especially in extreme and rapidly changing environments. Although any favoured gene complex in cyclic parthenogens could be frozen in successful clones independent of hybridization, we did not find similarly successful clones among parental species. We conclude that the emergence of the observed trait is linked to the production of novel recombined hybrid genotypes.

Full-text article:

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0140275

CHAPTER 5: THE ESTABLISHMENT OF HYBRIDS OF THE *D. LONGISPINA* COMPLEX EXPLAINED BY A MATHEMATICAL MODEL INCORPORATING DIFFERENT OVERWINTERING MODES.

Johanna Griebel, Margarete Utz, Joachim Hermisson, Justyna Wolinska

in preparation

The establishment of hybrids of the *D. longispina* complex explained by a mathematical model incorporating different overwintering modes.

Johanna Griebel[1,2], Margarete Utz[3], Joachim Hermisson[4], Justyna Wolinska[1,5]

[1]Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany

[2]Department of Biology II, Ludwig Maximilian University Munich, Grosshaderner Str. 2, 82152 Planegg-Martinsried, Germany

[3]Centre for Ecological and Evolutionary Studies, University of Groningen, Nijenborgh 7, 9747 AG Groningen, the Netherlands.

[4]Department of Mathematics, University of Vienna, Vienna, Austria

[5]Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Königin-Luise-Str. 1-5, 14195 Berlin Germany

Abstract

Intraspecific hybridization occurs frequently in animals. In a number of species, hybrids can proliferate and establish through parthenogenetic reproduction, even if sexual reproduction of hybrids is impaired. Regarding water fleas of the *Daphnia longispina* species complex, hybrids can express the same or higher fitness than parental species during the parthenogenetic part of the life cycle, whereas their success in sexual reproduction is reduced. Therefore, *Daphnia* hybrids would benefit from adaptations that enhance their overwintering survival as parthenogenetic lineages, thus avoiding the need to switch to the production of sexual resting eggs. Here, we constructed a mathematical model to examine the conditions that could explain the frequently observed establishment of hybrids in the *Daphnia longispina* species complex. Specifically, we compared the outcome of hybrid and parental species competition under different scenarios, including a reduction of sexual reproduction in hybrids, and simulating their enhanced survival over winter as asexual females. We also examined the influence of average annual temperatures on the establishment of hybrids. Our model shows that enhanced overwintering as asexual females can compensate for reduced success in sexual reproduction. This pattern holds true for lower annual temperatures, but at higher temperatures hybrids are unable to become established. Consequently, hybrids might become less abundant as temperatures rise due to climate change, resulting in reduced diversity and faster differentiation of the parental species.

Introduction

Hybridization and its implications for evolutionary and ecological processes have been widely discussed in recent decades (Abbott et al. 2013; Barton 2013; Burke and Arnold 2001; Rieseberg et al. 2007). Hybrids can display extreme phenotypes due to the combination of two parental genomes and therefore are sometimes able to establish in extreme environments. For example, hybrid Darwin finches on the Galapagos islands were favoured during a rare El Nino event, because the intermediate beak morphology of hybrids was best adapted to the smaller seed sizes caused by the event (Grant and Grant 1996). Hybrids of spadefoot toads display longer development times as tadpoles, a strategy beneficial during long dry periods (Pfennig 2007), whereas sunflower hybrids are able to establish on sand dunes, desert floors and salt marsh habitats (Rieseberg et al. 2007). Hybridization might result in parthenogenetic organisms (Delmotte et al. 2003); almost all parthenogenetically reproducing vertebrates have a hybrid origin (Avise et al. 1992). Therefore, by taking advantage of their capacity to reproduce parthenogenetically (e.g. aphids, Delmotte et al. 2003; freshwater snails, Johnson 2005; edible frogs, Semlitsch 1993; waterfleas, Spaak and Hoekstra 1995), hybrids might successfully establish despite their reduced abilities to reproduce sexually (Schwenk and Spaak 1997).

In cladoceran water fleas, *Daphnia*, hybrids are found worldwide (Hebert and Crease 1980; Keller et al. 2008; Yin et al. 2010). *Daphnia* mainly reproduce parthenogenetically, but under harsh conditions they form sexual dormant eggs (ephippia). During this sexual phase, interspecific hybrids can be produced. In the *Daphnia longispina* species complex such hybrids can successfully compete with their parental species during the parthenogenetic life cycle (e.g. Declerck and De Meester 2003; Seidendorf et al. 2007; Wolinska et al. 2007). However, hybrids suffer reduced efficiency of sexual reproduction; they have lower proportions of viable ephippia and lower hatching rates from these dormant eggs (Keller and Spaak 2004; Keller et al. 2007; Spaak et al. 2004). Unlike their parental species, hybrids therefore have a reduced ability to survive harsh conditions as dormant stages. Consequently, hybrid establishment relies mainly on parthenogenetic reproduction. In our recent experimental study, *Daphnia galeata* × *D. longispina* hybrids originating from several shallow lakes (i.e. strongly influenced by harsh winters) had an increased survival as parthenogenetic lineages under simulated winter conditions (4 °C, low food and 8:16 hours light-dark photoperiod, Griebel et al. 2015). Moreover, microsatellite screening of *Daphnia* communities sampled from the same lakes showed that hybrids are likely to survive winter as asexual females, in contrast to the parental species that survive mainly via sexually produced diapause eggs (Griebel et al. 2016). The ability to overwinter as asexual females can play an important role during recolonization of the water body in spring: individuals that are present first in the water column might quickly dominate the entire community, inhibiting the establishment of genotypes hatching from ephippia later in a season (De Meester et al. 2002; Lampert et al. 2010; Sommer et al. 1986). However, the interplay between reduced success in sexual reproduction on the one hand, and enhanced overwintering as asexual females on the other, has not been evaluated previously in terms of prospects for hybrid establishment.

We constructed a mathematical model, simulating a community consisting of two parental species of the *D. longispina* complex, F1-, F2-hybrids, and their species-specific backcrosses. First, we evaluated the influence of hybrids' reduced success in sexual reproduction on their establishment, using parameter values derived from published work (Keller and Spaak 2004; Keller et al. 2007). Second, we tested a scenario of hybrids' enhanced survival as asexual females during winter, adjusting the seasonal function for F1-hybrids during winter and using the survival data from our previous experimental study (Griebel et al. 2015). Seasonal changes in temperature and light, parameters strongly affecting *Daphnia* growth during the year, were simulated by applying a sinusoidal function from Scheffer et al. (1997). Third, different growth rates of the parental species and, fourth, differences in average annual temperatures, were evaluated for their influence on hybrid production and establishment.

Model

The aim of our model was to examine the conditions that could explain the frequently observed establishment of hybrids in the *D. longispina* complex. Specifically, we compared the outcome of hybrid and parental species competition under different scenarios such as reduced sexual reproduction in hybrids and an enhanced survival over winter as asexual females.

Individuals were divided into six classes: two parental species (j = 1 and j = 2), F1-hybrids (j = 3), backcrosses to respective parental species (j = 4 and j = 5) and F2-hybrids (j = 6). For each class the numbers of asexual individuals (A), sexual individuals (S) and resting eggs, ephippia (E), were modelled using the following differential equations:

$$\frac{dA_j}{dt} = r_j\, \sigma(t)\, A_j(t) \left(1 - \frac{\sum_{i=1}^{6}(A_i(t) + S_i(t))}{\sigma(t)K}\right) - s_j(t)A_j(t) + 2\, h_j(t)\, E_j(t) \qquad (1)$$

$$\frac{dS_j}{dt} = s_j(t)A_j(t) - m\, S_j(t) \qquad (2)$$

$$\frac{dE_j}{dt} = (1 - e_j) f \varphi_j(t) - h_j(t)\, E_j(t) \qquad (3)$$

The growth of asexual individuals is logistic with a specific intrinsic growth rate r_j for each class. The carrying capacity K is shared by all six classes, as well as asexual and sexual individuals. During two periods of the year (two weeks in May and September, days: 151 - 165 and 273 - 287, Keller and Spaak 2004) a fraction $s_j(t)$ of asexual individuals switch to sexual reproduction. Sexual individuals die at a fixed mortality rate m. At the beginning of April (days 119-120), ephippia (containing two embryos) hatch at a rate $h_j(t)$ and result in asexual individuals, mimicking a photoperiod-driven hatching of resting eggs (Stross and Hill 1968). Seasonal forces (seasonal cycle of light and temperature) were included by multiplying the carrying capacity K (affected by resource availability, changing with light conditions) and the growth rate r_j (affected by metabolic rates, changing with temperature) by a periodic function of time t (Fig. 1) with ε set to 0.7 as proposed by Scheffer et al. (1997):

$$\sigma(t) = \frac{1 - \varepsilon \cos\left(\frac{2\pi t}{365}\right)}{1 + \varepsilon} \qquad (4)$$

The production of ephippia was calculated using the harmonic mean developed by Caswell and Weeks (1986), which accounts for the fact that both females and males have to be present. The model was set up with equal fractions of males and sexual females for the different classes:

$$\varphi(t) = \frac{2\, k\, \frac{S_i}{2}(t)\, \frac{S_l}{2}(t)}{\frac{S_i}{2}(t) + \frac{S_l}{2}(t)} \qquad (5)$$

Here, *k* is the number of offspring produced per sexual encounter, which was set to one (Ebert 2005). $S_i(t)$ and $S_l(t)$ are the number of sexual individuals of class *i, l* $(i, l = 1 - 3)$ at time *t*. For simplification, sexual individuals of the backcrosses and F2-hybrids (classes j = 4, 5, 6, see above) do not take part in sexual reproduction. Ephippia of the hybrid (or backcross) class are produced by the two possible encounters of sexual females of one parental species (or hybrid) with males of the other parental species (or hybrid) and vice versa (Fig. 2). To simulate deviation from random mating, the sexual encounters were divided into individuals that only mate with individuals from their own class (fraction *c*) and individuals that mate randomly with any other individual (*1-c*). The fraction *c* was set the same for all three classes. These lead to the following equations:

$$\varphi_{j(t)} = \frac{\frac{1}{2}\, c\, S_j(t)\, c\, S_j(t)}{c\, S_j(t)} + \frac{\frac{1}{2}\,(1-c)S_j(t)\,(1-c)S_j(t)}{(1-c)\ \ (S_1(t) + S_2(t) + S_3(t))}$$

$$= \frac{S_j(t)\,(S_j(t) + c\left(S_1(t) + S_2(t) + S_3(t) - S_j(t)\right))}{2\,(S_1(t) + (S_2(t) + S_3(t))}\ for\ j = 1{,}2 \qquad (6)$$

$$\varphi_{3(t)} = \frac{(1-c)\,S_1(t)\,(1-c)S_2(t)}{(1-c)\,(S_1(t) + S_2(t) + S_3(t))} = \frac{(1-c)\,S_1(t)\,S_2(t)}{S_1(t) + S_2(t) + S_3(t)} \qquad (7)$$

$$\varphi_{4(t)} = \frac{(1-c)\,S_1(t)\,(1-c)S_3(t)}{(1-c)\,(S_1(t) + S_2(t) + S_3(t))} = \frac{(1-c)\,S_1(t)\,S_3(t)}{S_1(t) + S_2(t) + S_3(t)} \qquad (8)$$

$$\varphi_{5(t)} = \frac{(1-c)\,S_2(t)\,(1-c)S_3(t)}{(1-c)\,(S_1(t) + S_2(t) + S_3(t))} = \frac{(1-c)\,S_2(t)\,S_2(t)}{S_1(t) + S_2(t) + S_3(t)} \qquad (9)$$

$$\varphi_{6(t)} = \frac{\frac{1}{2}\, c\, S_3(t)\, c\, S_3(t)}{c\, S_3(t)} + \frac{\frac{1}{2}\,(1-c)S_3(t)\,(1-c)S_3(t)}{(1-c)\ \ (S_1(t) + S_2(t) + S_3(t))}$$

$$= \frac{S_3(t)\,(S_3(t) + c(S_1(t) + S_2(t)))}{2\,(S_1(t) + (S_2(t) + S_3(t))} \qquad (10)$$

The number of sexual encounters was multiplied by *f* (number of ephippia produced per day) the fraction of non-empty ephippia ($(1\text{-}e_j)$, equation (3)). Several scenarios were evaluated by solving the differential equations (1) – (3) numerically using Mathematica 10.3 (Wolfram Research Inc. 2015). If not stated otherwise, parameter values are as described in Table 1. Starting densities were set to 0.001 individuals per litre for each parental species and zero individuals for the other classes. Carrying capacity was set to 150 individuals per litre (Griebel et al. 2015).

Reduced sexual reproduction and enhanced overwintering in F1-hybrids

We evaluated how a reduced success in sexual reproduction influences the long-term (100 years) establishment of hybrids. Therefore, hatching rate h_j was set lower and the number of empty ephippia e_j was set higher in hybrids compared to parental species: 50 % and 12.5 %, respectively (Keller and Spaak 2004; Keller et al. 2007). The fraction of sexual individuals that mate within their own class was set at 75 % ($c = 0.75$). Growth rates were set the same for both parental species, F1-hybrids and backcrosses ($r_j = 0.35$ for $j \neq 6$). The growth rate for F2-hybrids was set lower assuming a hybrid breakdown ($r_6 = 0.3$, scenario A). Unfortunately, no data on fitness of F2-hybrids exist, as too few F2-hybrids are found in natural communities (Griebel et al. 2016; Keller et al. 2008) to be successfully established in laboratory cultures. We tested to what extent better overwintering success of hybrids as asexual lineages will change the establishment success of hybrids. Therefore, the ε of the seasonal force function (4) was set lower than in the original function (ε = 0.1, 0.175, 0.25 and 0.325) to increase the growth rate of hybrids (r_3) during winter (120 days; November till February, Fig. 1). These changes in ε result in 50 to 80 % higher growth rates of F1-hybrids at the minimum of the seasonal function (at day 365). In laboratory experiments, the growth rates during winter conditions (4 °C, low food and short photoperiod) were found to be seven times higher for hybrids compared to parental species (Griebel et al. 2015). We opted for more conservative values to exclude laboratory artefacts (e.g. experimental clones were sampled in spring, when only successful survivors were present). Additionally, the growth rate of F1-hybrids (r_3) was varied for different calculations (range: 0.30 to 0.35), to estimate whether the differences in winter survival may compensate for a generally lower fitness of asexual individuals in F1-hybrids. For example, in the laboratory hybrids had lower growth rates compared to parental species when raised at 20 °C (Weider and Wolf 1991). Analyses were run for 100 years.

Different growth rate scenarios for the parental species

In addition to scenario (A) in which both parental species have the same growth rates (which is unlikely in nature, Brede et al. 2009; Weider and Wolf 1991; Wolf 1987; Wolinska et al. 2006), we evaluated four other scenarios;

B) one parental species has a higher intrinsic growth rate (Weider and Wolf 1991); here: r_1=0.35 and r_2=0.3;

C) the intrinsic growth rates of the parental species alternate every second year (for example, because of changing parasite pressure, Wolinska et al. 2006); here: r_1=0.3 then 0.35 and r_2=0.35 then 0.3;

D) the intrinsic growth rates of parental species change within a year (because species might have reverse fitness at different seasonal conditions, Wolf 1987); here: switch at day 166, r_1=0.3 then 0.35 and r_2=0.35 then 0.3;

E) second parental species enters with a higher intrinsic growth rate, after the system has been dominated by a single parental species for 100 years (for example, due to eutrophication, Brede et al. 2009); here: r_1=0.3 and r_2=0.35).

In all scenarios, the consequences of the reduced success of sexual reproduction in F1-hybrids were evaluated for the likelihood of their establishment and how establishment success is changed by enhanced overwintering as asexual females. The growth rates of backcrosses (r_4 and r_5) were set to the average of the respective parental species and F1-hybrids. Growth rate of the F2-hybrids was set lower (r_6=0.3), whereas growth rate of F1-hybrids (r_3) was varied for different calculations (range: 0.30 to 0.35).

Different average annual temperatures

For all scenarios (A) – (E), the effect of differences in average annual temperature (differences of x °C) on the establishment of hybrids was tested by multiplying the growth rate of equation (1) by the parameter:

$$q = 2^{\frac{x}{10}} \tag{9}$$

The formula is derived from the temperature coefficient Q_{10}, which measures the change of biological processes induced by a temperature increase of 10 °C. The coefficient Q_{10} for *Daphnia* has been evaluated by Gorski and Dodson (1996) and was already applied in the predator-prey models of de Senerpont Domis et al. (2007) and Straile et al. (2001). Differences in temperature were tested for a change of $x = -5$ °C and $x = -3$ °C as well as $x = +3$ °C and $x = +5$ °C.

Results

Reduced sexual reproduction and enhanced overwintering in F1-hybrids

Analysis of the model shows that if sexual reproduction in F1-hybrids was reduced (i.e. 50 % lower hatching rate ($h_3 = 0.05$), 12.5 % more empty ephippia ($e_3 = 0.8$) compared to parental species ($h_{1,2} = 0.1$ and $e_{1,2} = 0.7$) and non-random mating), F1-hybrids were present at low numbers (< 20 *Daphnia* / L, proportion of 13 % , Fig. 3a). However, in the case of an enhanced survival of F1-hybrids as asexual individuals during winter, the number of established F1-hybrids increased under all simulated differences in the seasonal function (4) (i.e. 50 %, 60 %, 70 % and 80 % higher growth rates of F1-hybrids during winter compared to parental species, Fig. S1). Backcrosses as well as F2-hybrids were present in low numbers (Fig. S2). After an increase of the F1-hybrids' growth rate from 80 % during winter ($\varepsilon = 0.475$ in equation 4), F1-hybrids reached abundances of more than 50 % in the *Daphnia* community (Fig. S1). Therefore, the seasonal function with $\varepsilon = 0.1$ leading to 80 % higher growth rate during winter was applied for F1-hybrids and simulations were run for 100 years in all further analyses. If the general growth rate of hybrids was reduced by 12.5 % ($r3 = 0.31$) during the whole year, F1-hybrids were still detected, but below five *Daphnia* individuals per litre (Fig. 3c). They then coexisted with the parental species but were not able to dominate the system (Fig. 3c) as they did when their growth rate was equal to the growth rate of the parental species (Fig. 3b). Backcrosses and F2-hybrids were no longer present (Fig. S3c).

Different growth rate scenarios for the parental species

If sexual reproduction of F1-hybrids was reduced, they could not establish in two of the four further tested scenarios (i.e. additional to scenario A): in scenario B (where one parental species had a lower growth rate than the other parental species, Fig. 3d) and scenario E (where the second species with a higher growth rate was added after 100 years, Fig. 3m). In scenario E, F1-hybrids occurred for about 20 years but then vanished. When the growth rates of the parental species alternated, either within (scenario C) or between years (scenario D), F1-hybrids were detectable after ten years and quickly dominated the system (proportions of 60 – 75 %, Fig. 3 g, j). However, the smaller the difference in the average growth rates was between F1-hybrids and parental species in these scenarios (C, D), the smaller the numbers of hybrids (data not shown). In the case of parental species having altered growth rates within the year, the parental species with a higher growth rate in the second half of the year was more abundant in the system than the parental species that had a higher growth rate at the beginning of the year (Fig. 3j).

If the overwintering of F1-hybrids was enhanced (seasonal function with $\varepsilon = 0.1$ for 160 days: from November till February), then F1-hybrids occurred in all four scenarios of different growth rates for the parental species (B – E, Fig. 3). In scenario B (where one parental species had a lower growth rate than the other parental species, Fig. 3f) F1-hybrids only coexisted with the parental species, while in the other scenarios they dominated the system. The growth rate of F1-hybrids could only be reduced by 1.3 % ($r_3 = 0.347$) during the whole year in order to still facilitate their abundance compared to 12 % in scenario A (Fig. 3c). In scenarios C and D (growth rates of the parental species alternating within or between years, respectively), F1-hybrids dominated the system quicker (proportions of 85 % after 5 years) as is the case when overwintering was not enhanced via the seasonal function in F1-hybrids during winter (Fig. 3h, k). F1-hybrids were even able to coexist with the parental species when their growth rate was reduced by as much as 12 % ($r_3 = 0.31$) and 9.5 % ($r_3 = 0.32$), respectively (Fig. 3i, l). In scenario E (where the second species with a higher growth rate was added after 100 years), enhanced overwintering of asexual F1-hybrids facilitated their occurrence shortly after the addition of the second parental species and led to their dominance after about 20 years (66 %, Fig. 3n, the graph shows the 100 years after the new parental species was introduced). Furthermore, successful establishment of F1-hybrids was facilitated if their growth rates were reduced by up to 3 % ($r_3 = 0.34$). However, time until establishment was longer in such a case and the number of F1-hybrids decreased after 50 years to low numbers (Fig. 3o). In all scenarios, backcrosses and F2-hybrids occurred at low numbers (below ten individuals per litre) when overwintering of F1-hybrids was enhanced (Fig. S3).

Different average annual temperatures

Under lower average annual temperature (differences of -3 °C and -5 °C), F1-hybrids established faster compared to the previous analysis with x = 0 °C in scenarios B-E (Fig. 4). Only with a temperature decrease of -5 °C in scenario A F1-hybrids did not establish (Fig. 4a). In addition, the number of F2-hybrids was higher, whereas the number of backcrosses was lower, than in the analysis with x = 0 °C (Fig. S4). However, the effect of higher numbers of F1-hybrids during lower temperatures was small in scenarios C and D (alternating growth rates of parental species). In contrast, higher average annual temperatures (+3 °C and +5 °C) resulted in the failure of hybrids to establish in scenarios A, B and E. In scenarios C and D, numbers of F1-hybrids were low (Fig. 4). The numbers of F2-hybrids and backcrosses were lower, or these classes did not exist at all, when temperatures increased (+3 °C and +5 °C, Fig. S4).

Discussion

Our model indicates that reduced success in sexual reproduction in hybrids results in the situation that F1-hybrids cannot establish or establish only at low numbers, in three out of five simulated scenarios regarding the varying growth rates of parental species. Interestingly, in the remaining two scenarios (when the growth rates of the parental species alternate within and between years), F1-hybrids were able to outcompete the parental species and dominate the community. However, if only F1-hybrids had an enhanced survival as asexual females during winter, they dominated the system in all five tested scenarios of different growth rates of the parental species, and they were present even under a scenario of reduced growth rate during the whole year compared to their parental species. Lower average annual temperatures facilitated the successful establishment and dominance of F1-hybrids, while higher temperatures resulted in a failure of hybrids to establish.

Our model indicates that if hybrids' success in sexual reproduction is reduced and their growth rate is lower or equal to the growth rate of parental species, then F1-hybrids are present at low numbers only (scenarios A and E) or not at all (B). Reduced success in sexual reproduction and consequently lower production of ephippia, is therefore a clear disadvantage for hybrids, even though all classes survive winter as asexual females. In contrast, in another model, where different *Daphnia* species were not taken into account, overwintering asexual females were shown to always dominate the entire system (de Senerpont Domis et al. 2007). If the growth rates of parental species fluctuate between years or within years (scenario C and D) F1-hybrids become the most abundant in the community, even with lower success in sexual reproduction. Such success of F1-hybrids is caused by their higher overall average growth rate compared to the parental species during the year. Moreover, F1-hybrids' growth rate can still be lower than the maximum of each growth rate of the parental species (when parental growth rates vary within or between years) as long as it is above the overall average growth rate of each of the parental species. The overall average growth rate during the year for each class (parental species and F1-hybrids) therefore plays a major role in the establishment of hybrids.

Additionally, non-random mating had a larger impact on the establishment success of F1-hybrids than reduction in hatching rate and the number of empty ephippia, indicating that pre-zygotic barriers are more important than postzygotic ones. Studies on reproductive barriers in *Daphnia* have shown that time differences in production of sexual stages (Spaak 1995) as well as occupation of different habitats within a lake by the two parental species (Seda et al. 2007), both reduce encounter probability and thus, the possibility to hybridize. In nature, hatching rates are low and the proportion of empty ephippia is high in all classes of *Daphnia* (Keller and Spaak 2004; Spaak 1995). Therefore, differences in hatching rate and empty ephippia between F1-hybrids and parental species are unlikely to have a large impact. However, if overwintering as asexual females is enhanced in F1-hybrids, they are able to dominate the *Daphnia* community in all scenarios in our model. This is because enhanced overwintering leads to a generally higher average growth rate during the year. In that case the number of ephippia produced becomes less important.

The results of our model could explain patterns of *Daphnia* hybrid occurrence observed in nature. For example, although F1-hybrids were shown to have reduced success in sexual reproduction, in some years they have dominated the entire *Daphnia* community of lake Greifensee (Switzerland) (Keller and Spaak 2004; Keller et al. 2007). F1-hybrids first occurred in that lake in the 1950s, when the level of phosphorus rose and the second parental species *D. galeata* invaded the lake (Brede et al. 2009). This is consistent with scenario E in our model, where the introduction of a second species resulted in the quick establishment of F1-hybrids, in the case of hybrids having enhanced overwintering. Moreover, only small numbers of F2-hybrids and backcrosses were detected in Greifensee (Keller et al. 2007), consistent with the results of our model. Across several shallow lakes in Munich (Germany) F1-hybrids occurred together with only one parental species (Griebel et al. 2016; Yin et al. 2014). This indicates that hybrids do not need to be produced newly every season, consistent with the findings of our model. Indeed, F1-hybrids collected from these lakes have a higher overwintering survival probability than the parental species, as proven experimentally (Griebel et al. 2015). Moreover, genotype data of the aforementioned communities suggest that F1-hybrids survive winter mainly as asexual females (Griebel et al. 2016). Our model predicts the establishment, coexistence or dominance of F1-hybrids as long as the overwintering of F1-hybrids is enhanced, even if only one parental species is present in the long-term. Interestingly, only low numbers of F2-hybrids and backcrosses were detected across the described lakes (Griebel et al. 2016; Yin et al. 2014), again consistent with the results of the model.

In our model, average annual temperatures of lakes have an important effect on the occurrence of hybrids, when hybrids' overwintering is enhanced compared to parental species. Colder temperatures result in faster establishment and dominance of F1-hybrids, because only very low numbers of parental species survive as asexual females. Consequently, F1-hybrids reach high abundances before the parental species hatch from ephippia. Indeed, surviving as asexual females can lead to a competitive advantage in spring, when offspring of surviving *Daphnia* clones can quickly reproduce parthenogentically, and their offspring are born during an algal bloom (Sommer et al. 1986). This can then lead to later dominance (i.e. priority effects, Louette and De Meester 2007). Interestingly, in scenario A no F1-hybrids were detected when the average annual temperature was reduced by 5 °C suggesting that, below a certain temperature, no asexual females survive over winter. In this case, hatchlings from ephippia become the main colonization source in spring, resulting in a disadvantage to hybrids. Hybrids are also at a disadvantage at the opposite end of the temperature range, though for a different reason: high temperatures lead to large numbers of parental species surviving as asexual females and therefore hybrids lose their priority advantage and cannot establish. The model thus predicts that hybrids thrive at an intermediate temperature range (cold, but not too cold). The fact that higher temperatures lead to larger proportions of *Daphnia* surviving winter in an active form has been reported in other models (de Senerpont Domis et al. 2007; Schalau et al. 2008; Scheffer et al. 1997). As temperatures of lakes are rising throughout the world (Adrian et al. 2009), F1-hybrids may become less abundant in future. Indeed, we have observed previously that a successful hybrid clone (with experimentally proven enhanced overwintering as asexual females) was replaced in its natural habitat by the parental species after a warm winter (Griebel et al. 2015). However, more data would be required to examine the effect of temperature on hybrids' abundances in the field. If hybrids become less abundant in future, the possibility for introgression of genes between the parental species would be reduced, which could result in their faster genetic differentiation.

Overall, our model shows that hybrids' enhanced overwintering as asexual females might explain their frequently observed establishment and dominance in natural *D. longispina* communities. Even if F1-hybrids exhibit lower fitness than the parental species, enhanced overwintering can compensate for that. In general, differences in the overall average growth rates between parental species and hybrids play an important role in the establishment possibilities of hybrids. The success of hybrids' enhanced overwintering through asexual reproduction depends on average annual temperatures. Given that temperatures of lakes are rising, hybrids may become less abundant in future; this is because the number of parental species surviving winter will increase, consequently diminishing the advantages otherwise enjoyed by hybrids.

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Figures and tables

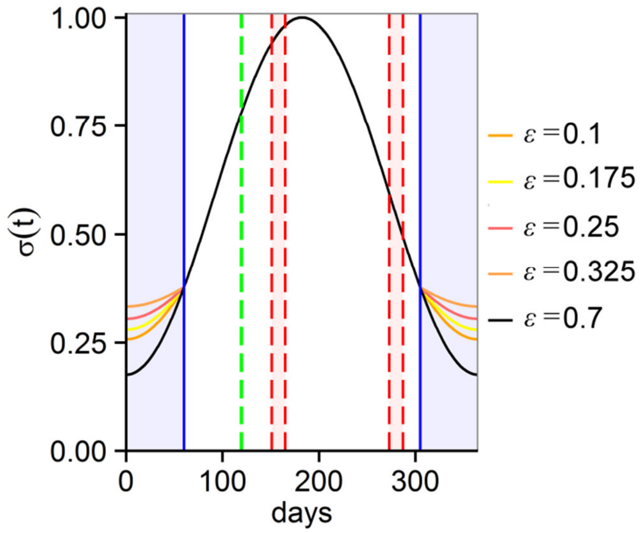

Figure 1. Adjustment of seasonal forces in *Daphnia* by *σ(t)*. The black line represents the standard curve for ε =0.7 (Scheffer et al. 1997), while the orange lines (ε = 0.1, 0.175, 0.25, 0.325) were used to obtain higher growth rates of F1-hybrids during winter (120 days from October to February). Blue vertical lines mark the winter period during which *σ(t)* differs for F1-hybrids. The red lines indicate the periods of sexual reproduction in early spring and autumn (May and September). The green line shows the hatching period from ephippia in spring (April).

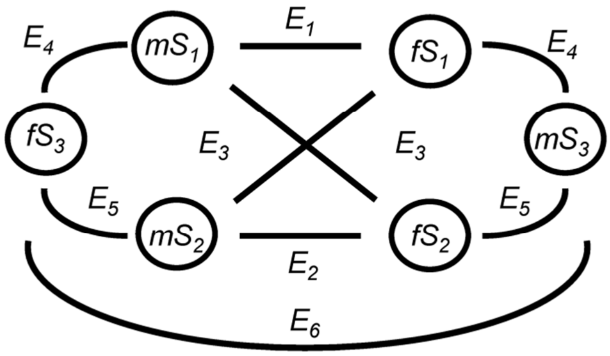

Figure 2. Possible encounters within and between the two parental species (class 1 and 2) and F1-hybrids (classes 3) during the phase of sexual reproduction resulting in ephippia of parental species, F1-hybrids, both backcrosses and F2-hybrids (E_j, j = 1-6). *m*: fraction of males, *f*: fraction of sexual females. S_1-S_3: sexual individuals of class 1-3.

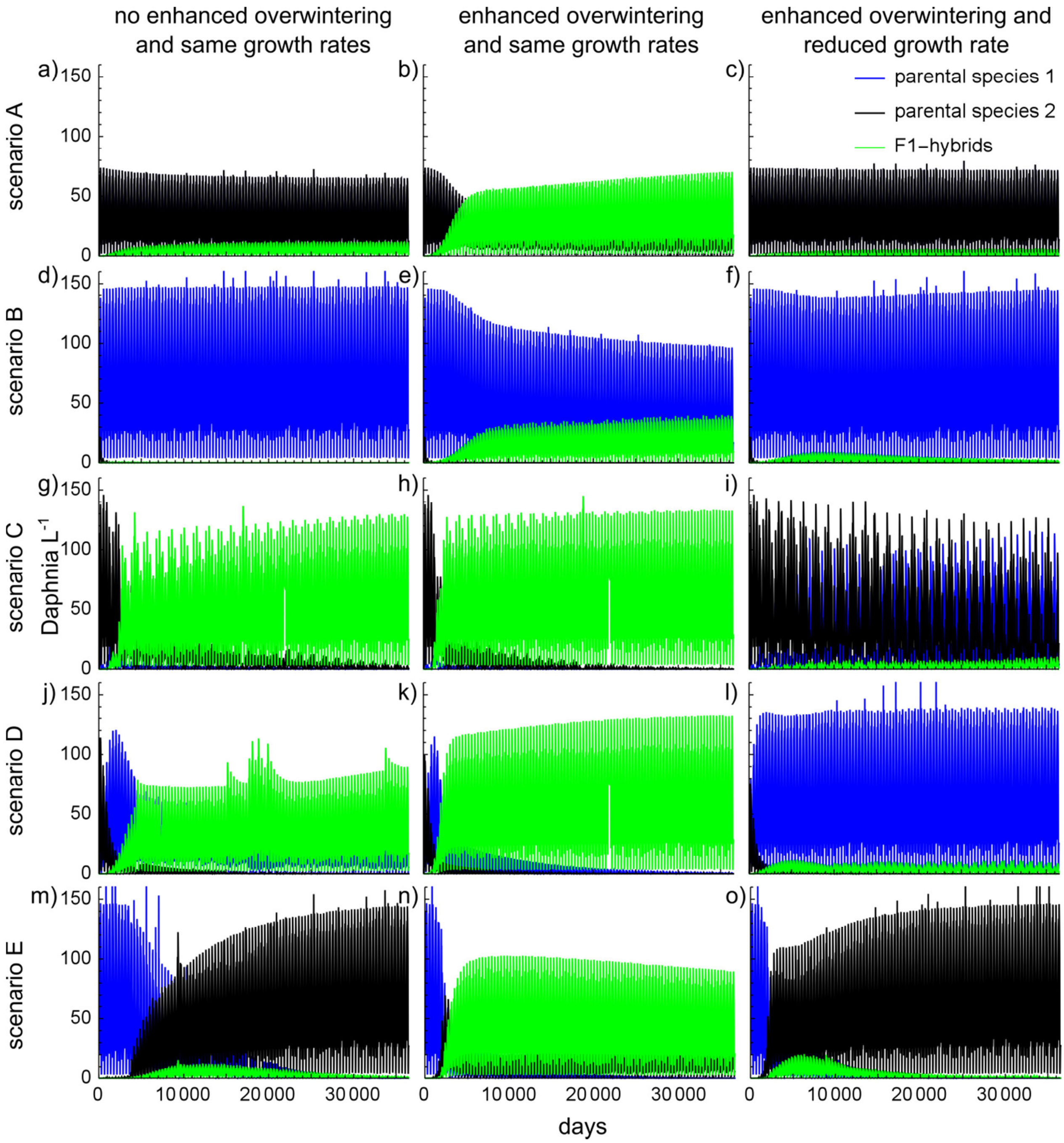

Figure 3. Numbers of individuals (asexual and sexual) of the two parental species and F1-hybrids over 36500 days (100 years) with F1-hybrids having reduced success in sexual reproduction (50 % lower hatching rates, 12.5 % more empty ephippia compared to parental species and non-random mating), with no enhanced overwintering for F1-hybrids *(left column*), with enhanced overwintering of F1-hybrids (ε=0.1 in $\varphi(t)$ for an 80 % higher growth rate during winter (*middle column*) and F1-hybrids having enhanced overwintering and reduced growth rate during the year (*right column*). The *top row* represents scenario A, where parental species have the same growth rate (curves of both parental species overlap), the *mid top row* represents scenario B, where parental

species 1 has a higher growth rate than parental species 2, the *mid row* represents scenario C, where the absolute growth rate of parental species alternates every two years, the *mid bottom row* represents scenario D, where growth rate of parental species alternates within the year and the *bottom row* represents scenario E, where the second species was introduced after 100 years, having a higher growth rate (graphs show the 100 years after parental species 2 had entered the system).

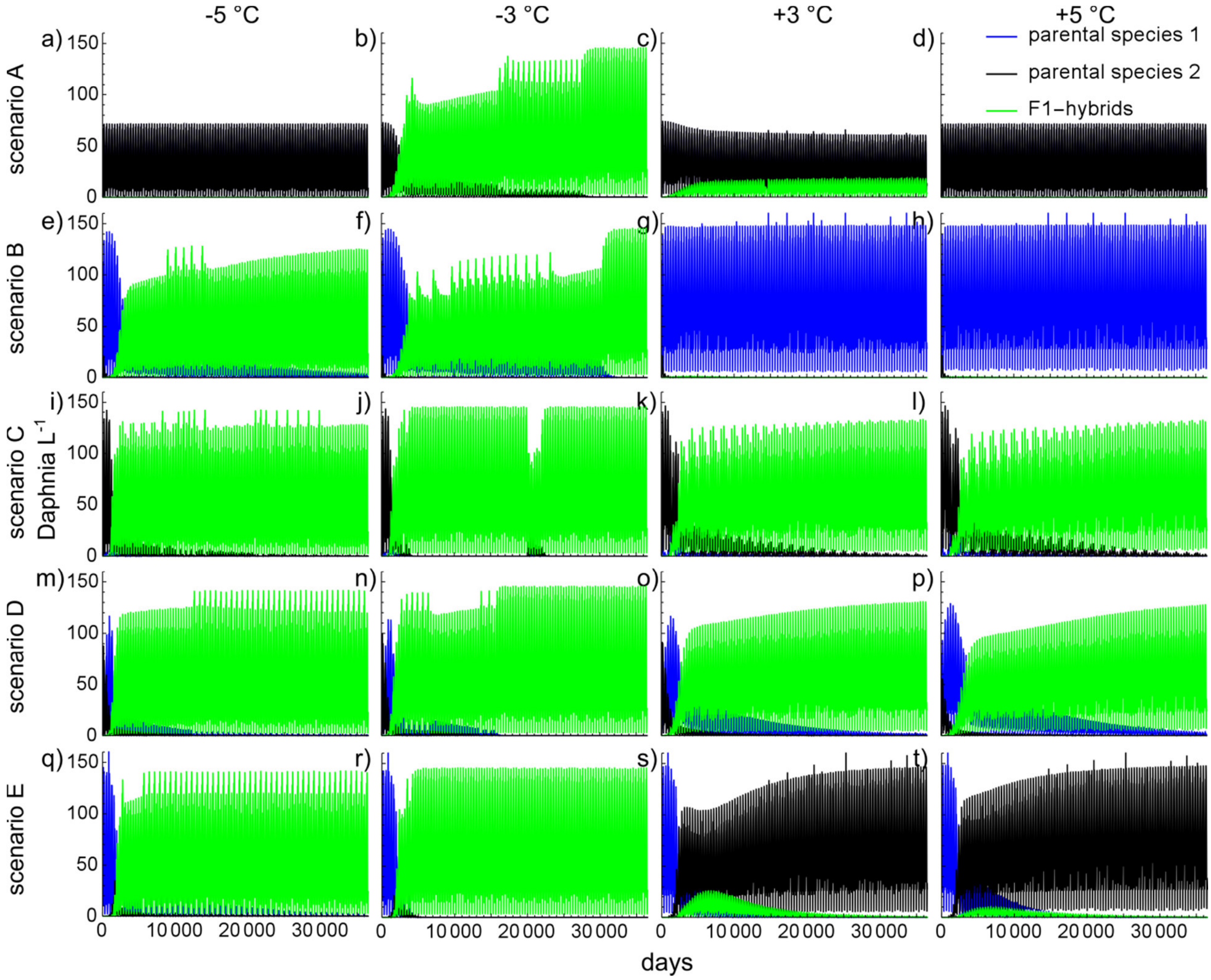

Figure 4. Numbers of individuals (asexual and sexual) of the two parental species and F1-hybrids over 36500 days (100 years) with F1-hybrids having reduced success in sexual reproduction (50 % lower hatching rates, 12.5 % more empty ephippia compared to parental species and non-random mating) and parental species differing in growth rates. The *ε* of the seasonal function of F1-hybrids differed during winter to simulate enhanced overwintering as asexual individuals for F1-hybrids compared to parental species (ε = 0.1 for an 80 % higher growth rate). The growth rate of F1-hybrids is 0.35. Differences in the average annual temperature (changes of -3 °C, -5 °C, +3 °C, and +5 °C) were applied for each scenario of different parental growth rates. The *top row* represents scenario A, where parental species have the same growth rate (curves of both parental species overlap), the *mid top row* represents scenario B, where parental species 1 has a higher growth rate than parental species 2, the *mid row* represents scenario C, where the absolute growth rate of parental species alternates every two years, the *mid bottom row* represents scenario D, where growth rate of parental species alternates within the year and the *bottom row* represents scenario E, where the second species was

introduced after 100 years, having a higher growth rate (graphs show the 100 years after parental species 2 had entered the system).

Table 1: Parameters of the model.

parameter	value	unit	description	Reference
m	0.15	day^{-1}	death rate	Schalau et al. (2008)
K	150	no. L^{-1}	carrying capacity	Griebel et al. (2015)
s_j	0.5	day^{-1}	fraction of individuals of class j switching to sexual reproduction	Keller and Spaak (2004)
f	0.14	day^{-1}	number of ephippia produced per day	Ebert (2005)
$e_{1,2}$	0.7		fraction of empty ephippia of class $j=1,2$	Keller and Spaak (2004)
e_3	0.8		fraction of empty ephippia of class $j=3$	Keller and Spaak (2004)
$e_{4,5}$	0.75		fraction of empty ephippia of class $j=4,5$	Keller and Spaak (2004)
e_6	0.85		fraction of empty ephippia of class $j=6$	Keller and Spaak (2004)
c	0.75		fraction of sexual individuals mating within own class	Keller et al. (2007)
$h_{1,2}$	0.1	day^{-1}	hatching rate from ephippia	Keller et al. (2007)
$h_{3,4,5,6}$	0.05	day^{-1}	hatching rate from ephippia	Keller et al. (2007)

Supplementary Figure

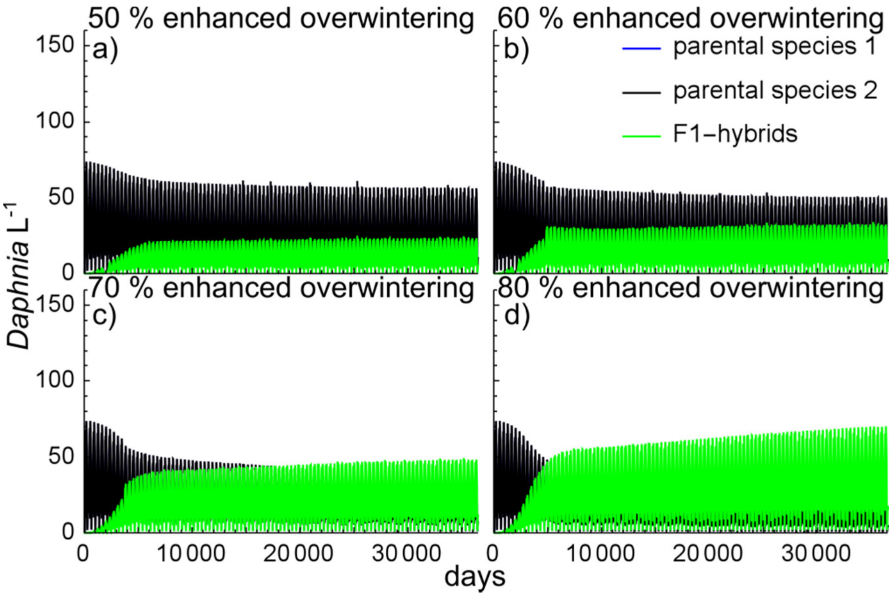

Figure S1. Numbers of individuals (asexual and sexual) of the two parental species and F1-hybrids over 36500 days (100 years) with F1-hybrids having reduced success in sexual reproduction (50 % lower hatching rates, 12.5 % more empty ephippia compared to parental species and non-random mating). Parental species and F1-hybrids have the same growth rates during the year ($r_{1,2,3}$ = 0.35) The ε of the seasonal function of F1-hybrids differed during winter to simulate enhanced overwintering of F1-hybrids compared to parental species: a) ε = 0.325 (50 % higher growth rate, b) ε = 0.25 (60 % higher growth rate), c) ε = 0.175 (70 % higher growth rate), d) ε = 0.1 (80 % higher growth rate). The curves of both parental species overlap.

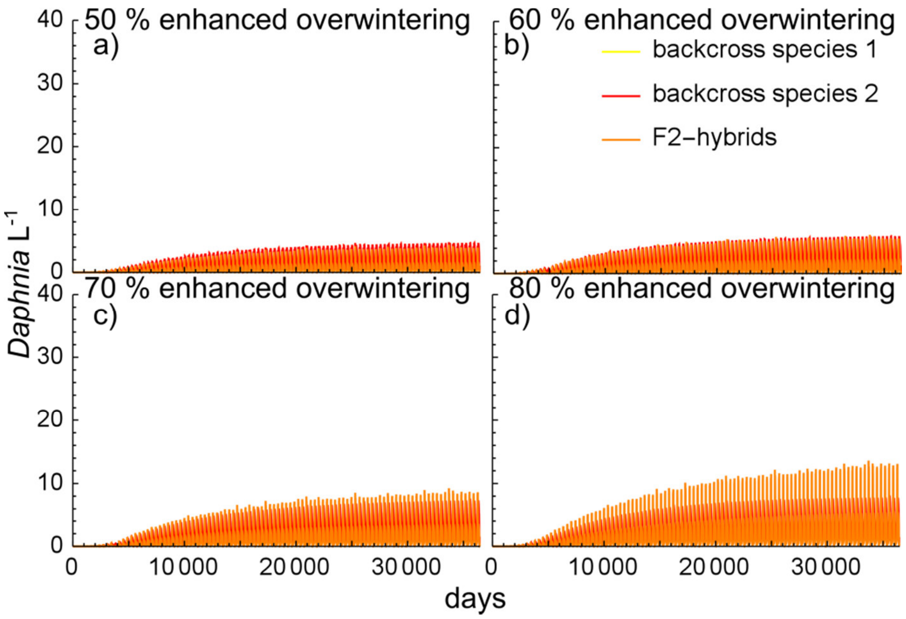

Figure S 2. Numbers of individuals (asexual and sexual) of the two backcrosses and F2-hybrids over 36500 days (100 years) with F1-hybrids having reduced success in sexual reproduction (50 % lower hatching rates, 12.5 % more empty ephippia compared to parental species and non-random mating). Parental species and F1-hybrids have the same growth rates during the year ($r_{1,2,3}$ = 0.35) The ε of the seasonal function of F1-hybrids differed during winter to simulate enhanced overwintering of asexual individuals compared to parental species: a) ε = 0.325 (50 % higher growth rate, b) ε = 0.25 (60 % higher growth rate), c) ε = 0.175 (70 % higher growth rate), d) ε = 0.1 (80 % higher growth rate).

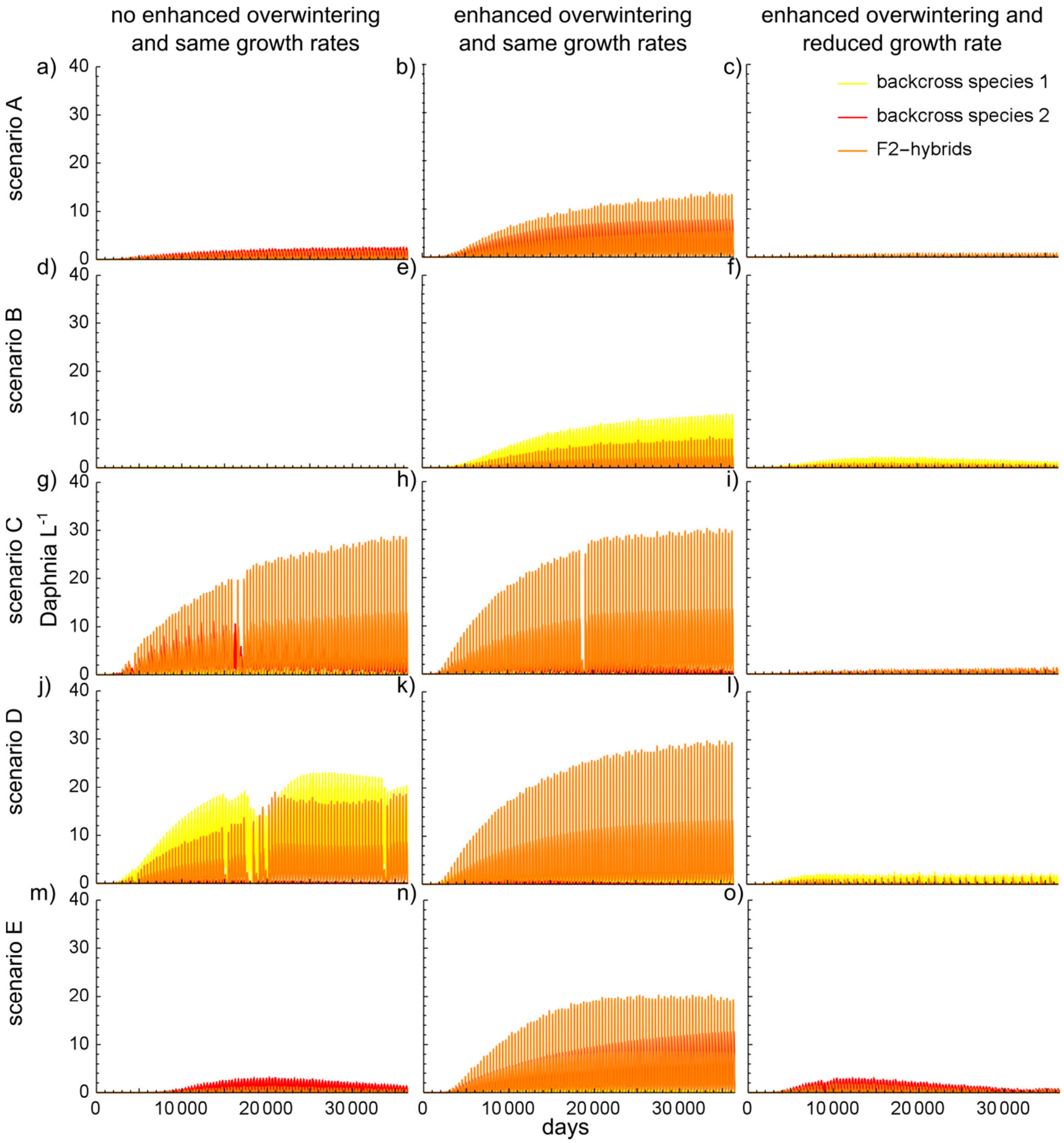

Figure S5. Numbers of individuals (asexual and sexual) of the two backcrosses and F2-hybrids over 36500 days (100 years) with F1-hybrids having reduced success in sexual reproduction (50 % lower hatching rates, 12.5 % more empty ephippia compared to parental species and non-random mating) with no enhanced overwintering for F1-hybrids *(left column*), with enhanced overwintering of F1-hybrids (ε=0.1 for a 80 % higher growth rate during winter, *middle column*) and F1-hybrids having enhanced overwintering and reduced growth rate during the year (*right column*). The *top row* represents scenario A, where parental species have the same growth rate (curves of both parental species overlap), the *mid top row* represents scenario B, where parental species

1 has a higher growth rate than parental species 2, the *mid row* represents scenario C, where the absolute growth rate of parental species alternates every two years, the *mid bottom row* represents scenario D, where growth rate of parental species alternates within the year and the *bottom row* represents scenario E, where the second species was introduced after 100 years, having a higher growth rate (graphs show the 100 years after parental species 2 had entered the system).

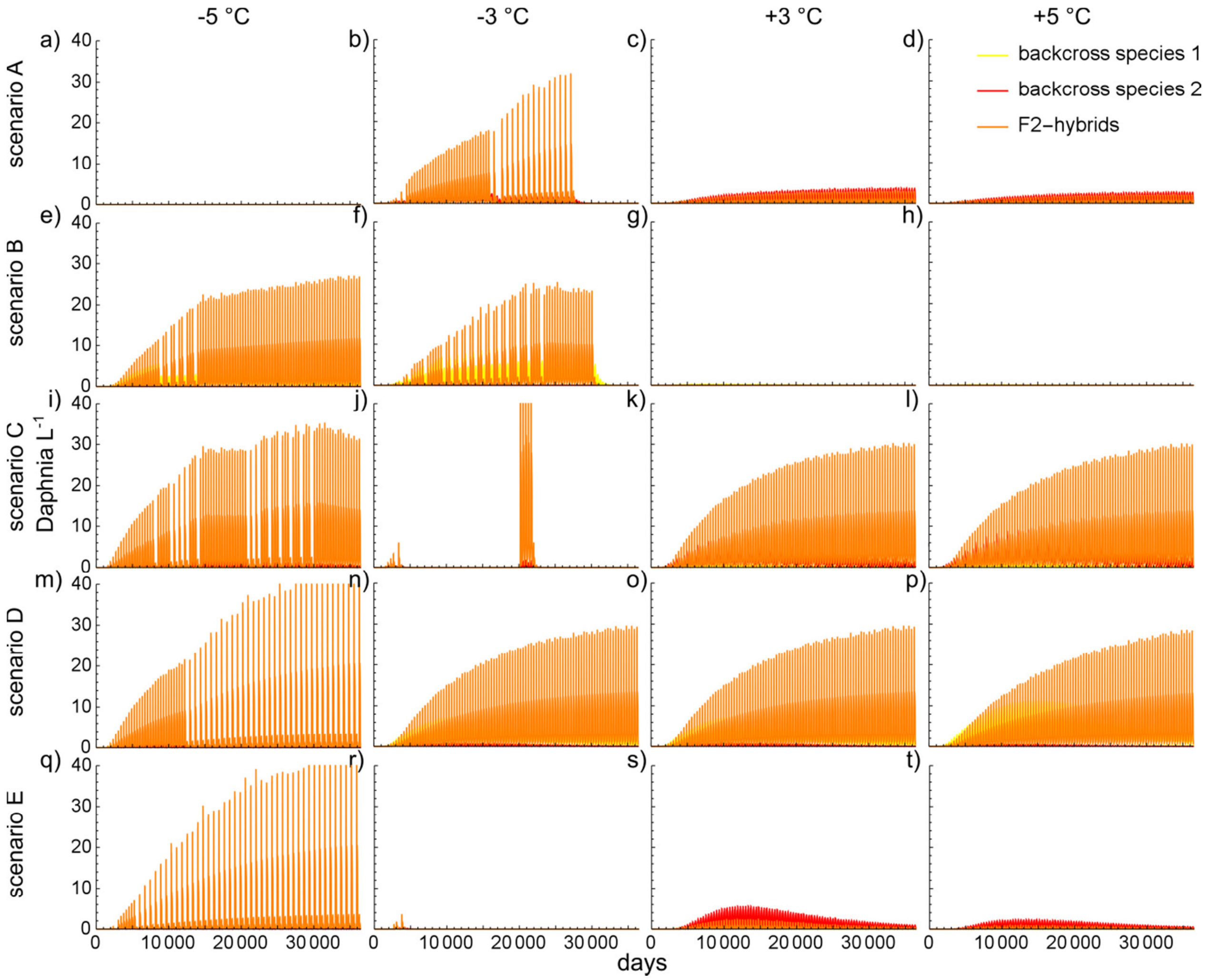

Figure S6. Numbers of individuals (asexual and sexual) of the two backcrosses and F2-hybrids over 36500 days (100 years) with F1-hybrids having reduced success in sexual reproduction (50 % lower hatching rates, 12.5 % more empty ephippia compared to parental species and non-random mating) and parental species differ in growth rates. The *ε* of the seasonal function of F1-hybrids differed during winter to simulate enhanced overwintering of asexual individuals compared to parental species (ε = 0.1 for an 80 % higher growth rate). The growth rate of F1-hybrids and backcrosses is 0.35, while F2-hybrids have a growth rate of 0.3. Differences in the average annual temperature (changes -3 °C, -5 °C, +3 °C, +5 °C) were applied for each different scenario of different parental growth rates. The *top row* represents scenario A, where parental species have the same growth rate (curves of both parental species overlap), the *mid top row* represents scenario B, where parental species 1 has a higher growth rate than parental species 2, the *mid row* represents scenario C, where the absolute growth rate of parental species alternates every two years, the *mid bottom row* represents scenario D, where growth rate of parental species alternates within the year and the *bottom row* represents scenario E, where the second species was introduced after 100 years, having a higher growth rate (graphs show the 100 years after parental species 2 had entered the system).

CHAPTER 6: GENERAL DISCUSSION

Discussion

This PhD thesis aimed to examine the role of hybridization in *Daphnia* during the colonization of newly opened habitats. Therefore, *Daphnia* communities of ten recently created shallow lakes (flooded gravel pits) were sampled in spring of six consecutive years and monthly during one year. Using microsatellite data, clonal diversity, migration patterns and differentiation between years were compared between parental species and their hybrids to examine how hybrids establish in spring. Three contrasting scenarios were tested: 1) recurrent hybridization events, 2), survival as asexual females during winter and 3) establishment of hybrids via migrants. Moreover, using different experimental set-ups (carrying capacity, temperature and crowding) a “successful hybrid” clone was compared with other clones of the parental species and their hybrids. Finally, observed parameters that might facilitate the establishment of hybrids were analyzed in a mathematical model.

Large numbers of F1-hybrids were found in four out of ten tested lakes (Chapter 2). These hybrids either coexisted with one of the parental species (three lakes) or dominated the community (one lake). Backcrosses and F2-hybrids occurred only in low numbers. Such different abundance patterns of hybrids of the *D. longispina* complex were observed already in other lakes in Europe (e.g. Keller et al. 2008; Yin et al. 2010). For example, the lake Greifensee (Switzerland) was dominated by F1-hybrids (proportion of ~80 %) during two sampled years, except for one sample where *D. galeata* dominated the *Daphnia* community (Keller and Spaak 2004). *D. longispina* was found only in small numbers (proportion of below 5 %). In these cases where only one parental species was detected, hybrids must have been produced earlier, when both parental species had been present, or the second parental species occurs only during short time periods and was therefore not detected.

In the spring samples of six consecutive years, hybrids had a higher heterozygosity and linkage disequilibrium compared to the parental species (Chapter 2). This observed pattern is likely the result of high proportions of parthenogenetic reproduction (Falconer et al. 1996; Fitzpatrick 2012). Moreover, hybrids showed a low genetic differentiation over time in contrast to the parental species. Therefore, hybrids are likely to establish in spring from asexual females that survived winter. In general, *Daphnia* show a mixed mode of how they survive winter: they produce sexual resting eggs but may also survive as parthenogenetic lineages (Lampert et al. 2010). Hybrids, however, have a lower sexual reproduction success. First, fewer resting eggs containing hybrids are produced, because mating between parental species is not random (Keller et al. 2007). Second, hybrids have lower hatching rates compared to the parental species (Keller and Spaak 2004). Therefore, it is likely that they would depend more on individuals that survive winter as asexual females. Surviving as asexual females can be advantageous in spring, as the offspring is directly born into the algae bloom, while individuals from resting eggs still need to hatch (Sommer et al. 1986). However, in lake Lerchenauer See the same *D. galeata* genotype and in lake Waldschwaigsee the same six *D. longispina* genotypes were detected repeatedly in different years (Chapter 2) indicating that parental species survive as asexual females as well. Also in other lakes (in Czech Republic) the parental species showed a high capability to survive winter as asexual females (Hamrová et al. 2011). This suggests that hybrids would need to have an enhanced survival as asexual females compared to the parental species during winter to successfully compete with them.

Indeed, in an experimental survey simulating winter conditions (low food and temperatures, short photoperiod) hybrids had a higher survival probability compared to the parental species (Chapter 4). Interestingly, the “successful hybrid” clone that dominated the entire *Daphnia* community in the Feldmochinger See in two consecutive spring samples had the highest survival probability in this survey. The “successful hybrid” and other hybrids used in the winter experiment produced more offspring during the experiment than the parental species. In another study simulating winter conditions, using *D. longispina* and hybrid clones collected from alpine lakes, only very low numbers of offspring were produced (Rellstab and Spaak 2009), which indicates that the hybrid clones sampled from the ten lakes used in this thesis have a special adaptation to winter conditions. The mathematical model in Chapter 5 shows that enhanced overwintering as asexual females indeed can facilitate the establishment and dominance of hybrids. Without hybrids’ enhanced survival a reduced sexual reproduction resulted in low numbers of hybrids during the year.

In general, clonal diversity was lower in hybrids compared to the parental species. Low numbers of clones have been found before in other hybrid systems that exclusively reproduce parthenogenetically (Johnson and Leefe 1999). One reason could be the earlier mentioned reduced success in sexual reproduction of hybrids (Keller and Spaak 2004; Keller et al. 2007; Spaak 1995). Alternatively, only certain hybrid genotypes might be successful, whereas others fail to establish. It is known that the relative fitness of *Daphnia* hybrids is influenced by the fluctuating environments (Löffler et al. 2004; Spaak and Hoekstra 1995; Wolinska et al. 2006), which might reduce the diversity of hybrids over time. For example, the winter period could select for asexual females that have an enhanced survival during winter. If the winter period is long and cold, hybrid genotypes that show an enhanced survival might be the only ones surviving. Indeed, clonal diversity of hybrids was lower with a higher severity of the winter (Chapter 2), which indicates that long and cold winter selects for hybrid genotypes able to survive as asexual females during such periods. However, fluctuations in the clonal diversity also suggest that new hybrid genotypes have to regularly hatch from resting eggs. Otherwise, due to selection of genotypes clonal diversity would be expected to decrease steadily over years (clonal erosion).

The model shows that colder average annual temperatures result in higher numbers of hybrids (Chapter 5). This was the result of only very few individuals of the parental species that survived as asexual females. In contrast, higher annual temperatures resulted in a loss of the advantage of hybrids' enhanced overwintering as asexual females, and they could not establish anymore. As temperatures of lakes are rising due to climate changes (Adrian et al. 2009), hybrids might become less frequent in *Daphnia* communities. Such a dependence of hybrid abundance on temperature was seen with the "successful hybrid", which first dominated the community of the Feldmochinger See in 2010 after a cold winter and vanished after a warm winter in 2012 (Chapter 4). However, there was no evidence that the proportion of hybrids was correlated with the severity of winter (Chapter 2). Regarding the latter one, six years might be a too short time period to observe such a correlation. Moreover, the abundance of hybrids can also depend on different environmental conditions (e.g. Declerck and Meester 2003; Weider and Wolf 1991; Wolinska et al. 2006), which might conceal such dependencies. In one study, for example, hybrids had higher growth rates than the parental species at 14 °C (Weider and Wolf 1991). The mathematical model in Chapter 5 even predicts that if the average growth rate of the hybrids is higher during the year than that of each parental species, they can establish, also if they have no enhanced survival as asexual females during the year. If the differences in the average growth rates are big enough, hybrids dominate the community. Therefore, it was tested if environmental parameters (e.g. phosphorus and nitrate level) can explain the abundance patterns of hybrids. It is known for the parental species that *D. galeata* occurs in rather eutrophic lakes, while *D.*

longispina occurs mainly in less eutrophic habitats (Brede et al. 2009; Keller et al. 2008). This was also the case in the here studied lakes (Chapter 3), however, abundance of hybrids was not correlated with any of the measured parameters.

The lakes sampled in this thesis are relatively young (the oldest lake was constructed in the 1930s). The relative fast development of an enhanced overwintering in hybrids might have been promoted by two mechanisms. First, as shown in Chapter 2, the winter period selects for hybrid genotypes expressing such an enhanced overwintering. Second, the combination of the parental genomes can facilitate a faster occurrence of novel or enhanced traits in hybrids (Burke and Arnold 2001).

Interestingly, as shown in Chapter 2, hybrids had mainly the mitochondrial haplotypes of *D. galeata*. Unidirectional hybridization can be due to premating barriers, unviability or lower fitness of such hybrids (Schwenk 1993). Previous studies of the *D. longispina* complex report both bidirectional (e.g. Gießler et al. 1999; Taylor and Hebert 1993) as well as unidirectional hybridization (Schwenk 1993). Therefore the here observed unidirectional hybridization (even though it is incomplete) might be caused by incompatibilities between interactions of nuclear DNA and mitochondrial DNA (Burton et al. 2006). If such interactions are broken up via hybridization, the energy supply by the mitochondria can be lowered. For example, in the marine copepod *Tigriopus californicus* ATP production was reduced in the F2-generation (Ellison and Burton 2006). This reduction in fitness was found to be dependent on temperature (Willett and Burton 2003) which might indicate why the hybrids found in our lakes have mainly *D. galeata* as mother. Overwintering in hybrids was enhanced and found to be the factor involved in a successful hybrid establishment. It is conceivable that the combination of two parental genomes in hybrids with mt-DNA of *D. galeata* provides the essential interaction to facilitate the overwintering and is selected for. This is supported by the fact that the only backcrosses we detected in the studied lakes were those with *D. galeata* (Chapter 2 and 3). If backcrosses have the parental mt-DNA haplotype as well as nuclear genes involved in the mitochondria energy production from the same parental species, fitness can be restored (Ellison and Burton 2008).

The relative young age of the lakes can facilitate migration of hybrids from other lakes. Normally gene flow between lakes, which have older *Daphnia* communities than the sampled here lakes, is low. In *Daphnia*, rapid population growth and local adaptation is suggested to lead to a monopolization of resources in the earlier stage of colonization (priority effects, De Meester et al. 2002). If *Daphnia* communities become older and therefore better adapted, it prevents newly arriving individuals from establishment. Moreover, large amounts of resting eggs in the sediment can provide a buffer against newly arriving genotypes (De Meester et al. 2002). But as the lakes sampled here are rather young they might still be providing a new habitat for invading *Daphnia* species and hybrids. However, hybrid populations of the lakes samples here were characterized by a high genetic differentiation between lakes and low numbers of migrants compared to the parental species (Chapter 2). In addition, microsatellite data from *Daphnia* community sampled monthly during one year, suggested that hybrids are rather locally produced (Chapter 3). Such a local production of hybrids was reported for several other lakes in Europe (Spaak 1997). The fact that fewer resting eggs containing hybrid genotypes are produced, is likely to explain the low migration rates of hybrids as *Daphnia* mainly disperse via ephippia transported by birds or wind (Bilton et al. 2001; Figuerola et al. 2005).

In Chapters 2-5, I present evidence that in the lakes sampled here, hybrids of the *D. longispina* species complex mainly survive winter as parthenogenetic females. Moreover, hybrids have developed an enhanced survival as asexual females helping them to overcome the reduced production of resting eggs. Enhanced survival of asexual females during cold periods was shown to be a possible explanation for the establishment and dominance of hybrids, as predicted by the mathematical model. However, if temperatures are rising the advantage of such a trait might be lost.

Future directions

The observed pattern of hybrids' enhanced overwintering found in the lakes studied in this thesis leaves several questions open. For example:

Is such an overwintering as asexual females a general enhanced trait of hybrids to compensate for reduced success in sexual reproduction? In fact, the scenario that hybrids have an enhanced overwintering as asexual females was tested for the first time. Further experimental studies and field studies with *Daphnia* hybrid genotypes from different lakes and different habitat types (shallow or deep lakes) could help to understand, if such a trait is generally enhanced or if this was a special case observed only for the lakes tested here.

What is the mechanism behind the enhanced overwintering as asexual females? In general it is assumed that low temperatures result in a down-regulation of the metabolic rate and therefore in an increased lifespan in *Daphnia*. In detail, low temperatures decrease the energy intake, assimilation and respiration of *Daphnia* (Burns 1968; Yurista 1999). Furthermore, during cold temperatures more highly unsaturated fatty acids have to be produced to maintain the cell membrane fluidity (Schlechtriem et al. 2006). Proteomic analysis of *D. pulex* raised under low temperatures revealed the up- and down-regulation of several protein groups, including up-regulation of vitellogenins (Schwerin et al. 2009). These proteins serve as vehicle for providing the developing embryos with essential resources. Therefore, higher expression of these proteins could be expected in the hybrids compared to the parental species, because hybrids produced more offspring than the parental species. Also, enzymes involved in extracellular digestion were found to be down-regulated in *D. pulex* under cold temperatures (Schwerin et al. 2009). Such a down-regulation actually would lead to decrease of protein digestion with lower temperatures. If observed, differences between hybrids and parental species in the down-regulation of these enzymes could be an explanation of the higher survival probabilities of hybrids. Furthermore, with the publication of the *D. pulex* genome (Colbourne et al. 2011), new possibilities arise also for the *D. longispina* complex to search for potential candidate genes and signaling pathways for the enhanced overwintering in hybrids.

Another explanation of an enhanced survival as asexual females could be the interaction between nuclear and mitochondrial DNA. The fact that hybrids mainly had mitochondrial haplotypes of *D. galeata* could be an indication of such an interaction. Therefore one would need to experimentally test if hybrids having mitochondrial haplotypes of *D. galeata* perform better under winter conditions than hybrids with *D. longispina* haplotypes. Also, if such differences in performance exist under colder temperatures it would be interesting to examine whether they vanish during warmer temperatures. It would be necessary to measure if energy production (e.g. ATP production) is higher in hybrids having mitochondrial haplotypes of *D. galeata* compared to hybrids with *D. longispina* haplotypes.

In the mathematical model of Chapter 5, some assumptions were made that are not always true in nature. This was a necessary simplification, as we wanted to concentrate on the overwintering patterns. For example, one assumption was that males and sexual females occur in the same frequencies. This is not always true in nature. First of all, one asexual female can only turn into one sexual female producing two resting eggs, while one asexual female can switch to the production of several males within one clutch (Ebert 2005). Furthermore in contrast to the induction of sexual females (Spaak 1995), the production of males is not species specific (Hobæk and Larsson 1990). Males are mainly induced by crowding (Banta and Brown 1929; Hobæk and Larsson 1990), while the induction of females depends also on temperature, photoperiod and food quality (Spaak 1995). Therefore, the establishment of hybrids can also depend on the frequencies and timing of male and sexual female induction. To examine the effect of differences in numbers and induction of sexual females and males, differential functions for males and females could be introduced, instead of using a differential equation for sexual individuals in general. Male production then could depend on the density of all *Daphnia* individuals. The female production could be simulated with a function of temperature and photoperiod. The dependencies on varying environmental cues could be set differently for the distinct species, which would allow examining the establishment of hybrids under different frequencies of males and females. Furthermore, on could simulate the migration of a new species that does not successfully establish, but takes part in hybrid formation through the production of males.

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AUTHORS CONTRIBUTION

Chapter 2:

S. Gießler and J. Wolinska designed the study. J. Griebel, J. Wolinksa, M. Yin collected the field data. J. Griebel analyzed the data and wrote the manuscript with the advice of J. Wolinska and S. Gießler.

Chapter 3:

S. Gießler and J. Wolinksa designed the study, M. Yin carried out the molecular work and J. Griebel collected the environmental dataset. M. Yin wrote the manuscript and analyzed the data with the help of S. Gießler and J. Wolinska.

Chapter 4:

J. Griebel, J. Wolinska and S. Gießler designed the experiments. M. Yin, J. Griebel and J. Wolinska collected the field data. J. Griebel, M. Poxleitner and A. Navas Faría performed the experiments. J. Griebel analyzed the data. J. Griebel and J. Wolinska and S. Gießler wrote the manuscript.

Chapter 5:

J. Griebel and M. Utz developed the mathematical model with the advice from J. Hermisson and J. Wolinska. J. Griebel performed the analysis of the model. J. Griebel wrote the manuscript with the help of J. Hermisson, M. Utz and J. Wolinska.

STATUTORY DECLARATION AND STATEMENT

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

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Hiermit erkläre ich, *

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*) Nichtzutreffendes streichen

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CV

Personal Data

Education

2012 – present	PhD candidate at the Department of Evolutionary Ecology of the Ludwig-Maximilian-University Munich Thesis title: **“The role of hybridization in the colonization of newly opened habitats”** Supervisor: Prof. Dr. Justyna Wolinska
2005 – 2011	Study of Biology (Zoology, Cell Biology, Forensic Medicine and Mathematics) at the Christian Albrechts University Kiel Diploma thesis: **“Genetic structure of the roe deer (*Capreolus capreolus*) in the cultural landscape of Poland”**

Professional experience

2014 – present	PhD student at Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin
2012 – 2014	PhD student at Biology Department Ludwig-Maximilian-University Munich

Conferences

2015	Symposium “Population and Evolutionary Genomics” of DFG Research Unit FOR 1078, Starnberg, Germany. Poster: **“Comparison of population genetic parameters of parental and hybrid *Daphnia* from the *D. longispina* complex: differences in diversity, genetic differentiation, gene flow and the overwintering strategies”,** <u>J. Griebel</u>, S. Gießler, M. Yin, J. Wolinska
2014	10th Symposium on CLADOCERA, Lednice, Czech Republic. Oral talk: **“Why a hybrid outcompeted its parental species? A story about a “super clone”**, <u>J. Griebel</u>, S. Gießler, M. Poxleitner, A. Navas Faría, M. Yin, J. Wolinksa
2014	FOR Meeting 1078 Meeting, Munich, Germany. FOR 1078 Meeting/Symposium “Population Genomics”, Oral talk: **“The role hybridization in the colonization of newly opened habitats”,** <u>J. Griebel</u>
2013	106th Annual Meeting of the German Zoological Society, Munich, Germany. Oral talk: **“Can a hybrid outcompete its parental**

	species? – a story about a "super clone" (*Daphnia galeata* × *longispina* hybrid)", J. Griebel, M. Poxleitner, A. Navas Faría, S. Gießler, J. Wolinksa
2013	14th Congress of the European Society for Evolutionary Biology, Lisabon, Portugal. Poster: **"Can a hybrid outcompete its parental species? – a story about a "super clone" (*Daphnia galeata* × *longispina* hybrid)"**, J. Griebel, M. Poxleitner, A. Navas Faría, S. Gießler, J. Wolinska
2013	FOR 1078 Meeting/Symposium "Population Genomics", Oral talk: **"The role hybridization in the colonization of newly opened habitats"**, J. Griebel
2012	105th Annual Meeting of the German Zoological Society, Konstanz, Germany. Oral talk: **"Can a hybrid outcompete its parental species? – a competition experiment with a "super clone" (*Daphnia galeata* × *longispina* hybrid)"**, J. Griebel, M. Poxleitner, S. Gießler, J. Wolinska
2012	FOR 1078 Spring meeting, Munich, Germany. Oral talk: **"The role hybridization in the colonization of newly opened habitats"**, J. Griebel

Workshops and Courses

2014	"Advanced statistics: Selected multivariate methods in R", IGB Berlin, Dr. Gabriel Singer
2013	"Models in Ecology and Evolution", LMU Munich, Dr. Margarete Utz
2012	"Evolutionary Biology Workshop in the Alps", University Lausanne, Arolla (Switzerland), Prof. Tadeusz J. Kawecki

Peer-reviewed Publications

J. Griebel, S. Gießler, M. Yin, J. Wolinska (2016): **Parental and hybrid *Daphnia* from the *D. longispina* complex: long-term dynamics in genetic structure and significance of overwintering modes.** Journal of Evolutionary Biology 29:4

J. Griebel, S. Gießler, M. Poxleitner, A. Navas Faria, M. Yin, J. Wolinska (2015): **Extreme environments facilitate hybrid superiority – the story of a successful *Daphnia galeata* × *longispina* hybrid clone**. PlosOne 10:e0140275

M. Yin, S. Gießler, J. Griebel, J. Wolinska (2014): **Hybridizing *Daphnia* communities from ten neighboring lakes: spatio-temporal dynamics, local processes, gene flow and invasiveness.** BMC Evolutionary Biology 14:80

Manuscripts in preparation

M. Yin, X. Ma, S. Gießler, A. Petrusek, S. Gießler, J. Griebel, W. Hu, J. Wolinska (in preparation): **Cytonuclear diversity and shared mitochondrial haplotypes among *Daphnia galeata* populations separated by twelve thousands kilometres.**

J. Griebel, M. Utz, J. Hermisson, J. Wolinska (in preparation): **The establishment of hybrids of the *D. longispina* complex explained by a mathematical model incorporating different overwintering modes.**

M. Saebelfeld, L. Minguez, J. Griebel, M. O. Gessner, J. Wolinska (in preparation): **Rapid increase in dissolved organic carbon drives oxidative stress and fitness impairments in *Daphnia*.**