# Evolutionary and ecological consequences of interspecific hybridization in cladocerans

K. Schwenk and P. Spaak*

*Netherlands Institute of Ecology, Centre for Limnology, Rijksstraatweg 6,*
*NL-3631 AC Nieuwersluis (The Netherlands), Fax +31 2943 2224; e-mail schwenk@nioo.nl and*
*spaak@mpil-ploen.mpg.d400.de*

**Abstract.** The evolutionary process of interspecific hybridization in cladocerans is reviewed based on ecological and population genetic data. The evolutionary consequences of hybridization, biogeographic patterns and fitness comparisons are analyzed within the conceptual framework of theories on hybridization. Among species of the *D. longispina* complex no interpopulational transition zones (hybrid zones) have been detected, but rather patchy distributions of hybrids and parentals have been found. Hybrids occur across broad geographic ranges and can be more abundant than parental species. Due to asexual reproduction (ameiotic parthenogenesis), hybrid breakdown can be avoided, and hybrids can even (temporarily) combine advantageous traits of both parental species. Evolutionary consequences may arise from repeated backcrossing, which in some cases results in introgression and patterns of reticulate evolution.

**Key words.** Natural interspecific hybridization; introgression; parthenogenesis; life histories; population genetics; *Daphnia longispina* complex.

> *Those forms which possess in some considerable degree the character of species, but which are so closely similar to some other forms, or are so closely linked to them by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us*
>
> (Charles Robert Darwin 1859)

## Introduction

A fundamental aim of evolutionary biology is to provide proximate and ultimate explanations for the variation and diversity of organisms in space and time. Because of the complex interactions of different processes like genetic drift, mutation and selection, it is difficult to estimate the significance of one specific evolutionary factor. Compared with these evolutionary processes, interspecific hybridization provides a prominent window to the genetic changes in populations[51,63]. Hybrids possess a combination of genes from two different (parental) gene pools. In contrast to the relatively slow rate of change in gene frequencies due to selection or genetic drift, a hybridization event can be a *dramatic* process. $F_1$ hybrids differ markedly from their parental species since, in a single generation, two originally separated gene pools may create significantly more combination of genes than recombination within a given species. Comparing parental and hybrid taxa offers the possibility to test evolutionary theories of selection, adaptation and gene flow[51,52].

Hybridization is very common among plant species, for example Stace[116] argued that 50 to 70% of extant angiosperms are the products of hybridization between species of the same or different genera (see also ref. 135). In contrast to the traditional view, which suggests the rare presence of hybrids in the animal kingdom[40,80,91,133], recent investigations have revealed an increasing number of hybridization events among many animal taxa[20,53,76,89,129].

The establishment of hybrids among sympatric species depends on both the possibilities for interspecific cross-fertilization (e.g. spatial/temporal synchronization, field frequencies, mate choice) and its success in a specific habitat (i.e. relative fitness of hybrids). Hybridization has frequently been considered as a race between fusion and speciation, with the outcome depending on the fitness of hybrids and the initial level of positive assortative mating[51,52]. Studies of natural interspecific hybridization have addressed mainly five issues:

1) origin of hybridization (sympatric or allopatric, biogeographic pattern),
2) hybrid zone dynamics (maintenance of hybridization, stability of hybrid zones),
3) genetic and evolutionary consequences of hybridization (introgression, reticulate evolution, phylogeny),
4) speciation and genetic/reproductive isolation (species concept, assortative mating, mating barriers), and
5) fitness comparisons (habitat associations, ecological isolation).

In the following, we first provide a framework of theories on interspecific hybridization and then summarize patterns of hybridization among cladocerans. Furthermore, we call attention to recent work on the *Daphnia longispina* complex, which comprises six commonly studied species and six hybrids (fig. 1). Several inquiries

---

* Present address: Abteilung Ökophysiologie, Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön (Germany).

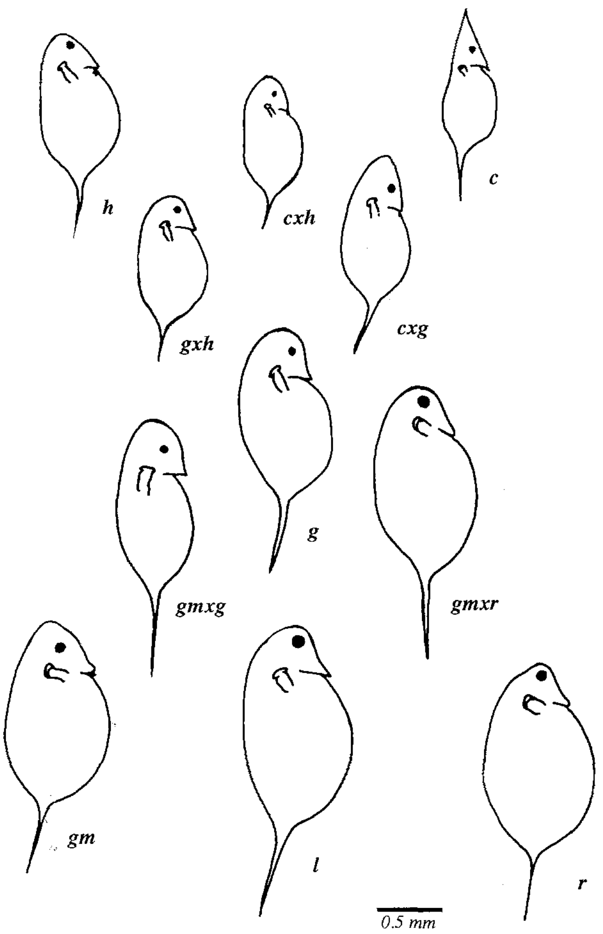

Figure 1. Typical morphs of the *D. longispina* species complex redrawn from Flößner and Kraus[38], Flößner[37], Taylor and Hebert[123] and Derek Taylor, pers. commun. The entire complex comprises five species (**l**: *D. longispina*, **g**: *D. galeata*, **c**: *D. cucullata*, **h**: *D. hyalina*, **r**: *D. rosea*), one sub-species (**gm**: *D. galeata mendotae*) and six interspecific hybrids (**gxh**: *D. galeata x hyalina*, **cxh**: *D. cucullata x hyalina*, **cxg**: *D. cucullata x galeata*, **gmxg**: *D. galeata mendotae x galeata*, **gmxr**: *D. galeata mendotae x rosea* and *D. longispina x galeata*, which is not displayed).

in the genus *Daphnia* have focused on taxonomy, population genetics, life history evolution and phylogeny. Using genetic and ecological data, we address the following issues:

1) the population structure of species complexes,
2) biogeographic patterns,
3) fitness comparisons, and
4) evolutionary consequences of interspecific hybridization.

In general, we aim to compare and contrast interspecific hybridization among *Daphnia* species with other animal species complexes, and discuss these results in the context of models on hybrid maintenance.

## Conceptual framework of theories on interspecific hybridization

The majority of theoretical work on hybrid zones has concentrated on hybrid zone origin (secondary contact vs primary intergradation), and on the maintenance of hybridization and speciation[1,5,11,12,19,51,52,63,94]. According to their basic assumptions, models of hybrid zone maintenance can be grouped into two classes. One group of hypotheses is based on the assumption that the relative fitness of genotypes is largely determined by ecological factors, hence selection regimes vary along spatial gradients (e.g. bounded hybrid superiority model, mosaic models, gradient and ecotone models[34,94]). Alternatively, tension zone models assume no ecological components responsible for selection processes, but only consider endogenous factors, such as genetic incompatibilities of parental genomes[11].

The **bounded hybrid superiority model** assumes that parentals are adapted to different environments, but that hybrids are more successful in certain habitats than either parent[94,95]. The location of these habitats is responsible for species and hybrid distributions, because relative fitness is mainly determined by ecological factors. Botanists have stressed that hybridization is often associated with unstable and rapidly changing or disturbed habitats[2,46,61,117].

**Gradient and ecotone models** assume that environmental gradients cause differences in selection. Parentals might be adapted to each extreme habitat, whereas the hybrid is superior in an intermediate situation (habitat). Thus, hybrid maintenance is dependent on selection gradients and the extent of dispersal[34]. Since environmental factors do not always change along gradients, but also among discrete patches, **mosaic models** have been proposed[53]. For example, field crickets (*Gryllus pennsylvanicus*, *G. firmus* and their hybrids) are patchily distributed within a 'hybrid zone', according to the spatial distribution of certain soil types; hence, the broad hybrid zone is actually a patchwork of populations[53,101]. Parental species might differ in fitness among patches, thus hybrid distribution is largely determined by dispersal and the relative fitness of parentals and hybrids. The structure of the hybrid zone will be mosaic only in environments where dispersal distances are small compared with patch size. If dispersal distances are large compared with patch size, then a gradient pattern (not a mosaic) would be expected[53]. Several cases of hybrid taxa which are ecologically differentiated have been found, e.g. species distributions according to abiotic factors such as soil type, altitude or moisture[15,101] and biotic factors, such as host plant differences[113].

In contrast to the models mentioned above, **tension zone models** assume that environmental factors are not involved in the maintenance of hybrid zones[11]. Theoretical and empirical studies have shown that a dynamic equilibrium, which results from a balance between natural selection and dispersal, can account for hybrid zone maintenance[11,12]. Selection operates through reduced fitness of hybrids, e.g. due to (partial) genetic incompatibility of parental genomes. Several examples have been reported where hybrids are sterile[66], have reduced viability[10], or have relatively higher embryonic mortality[120]. One of the strongest arguments supporting tension zone models is the observation of concordant clines among independent characters (such as morphology, nuclear and mitochondrial DNA markers). Such patterns are difficult to explain solely by selection, since it implies that all characters exhibit an identical response to environmental factors.

In addition to numerous examples of narrow hybrid or tension zones, some examples are known where parentals and hybrids occur in sympatry across a broad geographic scale[60,69]. Although hybridization can occur on a continental scale in these cases, parental taxa are able to maintain their distinctness, probably through ecological differentiation. Whether hybrid zone maintenance and geographical patterning are adequately explained by ecological factors or by an equilibrium between selection against hybrids and dispersal is an open question. Since tension zones are not linked to ecological factors, they presumably are able to move[73]. Hewitt and Barton[65] pointed out that the movement of tension zones and their tendency to become 'frozen' along natural barriers complicates the discrimination between the hypotheses because the association of ecotones with species and hybrid distributions could be coincidental. Although it is not our intention to evaluate different theories of hybrid zone maintenance, we would like to emphasize their significance in studying the relationship between ecological parameters and selection in hybrid zones, e.g. through fitness comparisons.

Besides studies on the maintenance of hybridization, several, mainly empirical, inquiries focused on evolutionary and genetic consequences of interspecific hybridization, such as the directionality of hybridization, phylogenetic relationships and introgression. In line with Rieseberg and Wendel[103], we define introgression as gene exchange between species, subspecies, races, or any other set of differentiated population systems. Although we do not want to discuss the different definitions and ways of detecting introgression, we would like to stress that the term introgression has been used for several different phenomena, and the unambiguous detection of interspecific gene exchange is not straightforward[3,51,103]. In general, the process of introgression comprises cytoplasmic introgression (mitochondrial or chloroplast DNA), and nuclear DNA introgression. These processes are presumed to be important evolutionary factors, since they might lead to an increase in genetic diversity, or to the origin or transfer of adaptations (for review see ref. 103). Furthermore, introgression has substantial consequences for phylogenetic relationships[5], for relative fitness values[81,82] and for adaptive properties of introgressants, in particular in disturbed or changing environments where new ecological niches become available[118]. Introgression has been studied extensively in plant species (for reviews see refs 61, 103) and during the last decade also has gained prominence in animal studies[7,8,21,35,39,47,54,90,100,106,107,114,120,125]. Several investigations of introgression of mitochondrial and nuclear DNA in animals have revealed patterns of differential introgression (i.e. discrepancies between nuclear and cytoplasmic DNA introgression). In general, more examples are known where cytoplasmic gene flow between taxa is greater than nuclear gene flow. Although the ultimate causes for this discrepancy are not understood, several hypotheses have been proposed. For instance, selection against either alien nuclear[13,100], or alien cytoplasmic genes[102], or asymmetrical reproductive isolation[7] has been proposed. If one disregards the mechanism as such, and focuses on the evolutionary consequences, in particular reticulate patterning of phylogenies, then several additional questions can be raised[5]. For example, do ecological, genetical or historical factors determine the spatial distribution of introgression? Is introgression asymmetric or bidirectional? How long has introgression proceeded: a few or many generations?

## Hybridization in cladocerans

Despite extensive empirical as well as theoretical studies of hybrid zones among animals, in particular in terrestrial habitats (e.g. refs 11, 45, 51, 52, 64), very little information has been derived from freshwater habitats, especially lakes. Hybridization among aquatic organisms has only recently been studied[8,15,25,29,39,74,106,107]. Terrestrial habitats frequently show a gradient in ecological parameters like temperature, humidity and vegetation structure. Hybrids tend to occur in intermediate environments and can exist either in sympatry or parapatry with the parental species. In contrast, freshwater habitats, particularly lakes and ponds, are characterized by a patchy to linear spatial distribution, by homogeneity in abiotic and biotic factors, and by distinct 'island-like' isolation from each other.

Among aquatic organisms, members of the microcrustaceans (Anomopoda, Ctenopoda, Haplopoda and Onychopoda), exhibit several unique features related to the study of interspecific hybridization. Species in this group reproduce sexually and asexually (via obligate or cyclic ameiotic parthenogenesis), therefore $F_1$ hybrids are able to circumvent possible deleterious effects of reduced sexual fertility by propagating asexually. Zooplankton species are often confronted with low dispersal rates but relatively stable environments. Species which produce resting eggs can be dispersed via other organisms or wind. However, they face two basic problems: first, dispersal is passive, and is dependent on the availability of potential vectors and second, the likelihood of reaching a suitable new habitat is low. Asexual reproduction and relatively short generation times (e.g. the genus *Daphnia*) facilitate the detailed study of life history variation and quantitative genetics (e.g. refs 32, 115, 130). These characteristics allow fitness comparisons and the possibility of testing assumptions of hybrid maintenance with regard to selection and ecological differentiation[112, 130].

Among cladocerans, interspecific hybridization has been investigated among species of *Simocephalus*[50], *Pleuroxus* and other chydorids[42, 108], *Bosmina*[26, 27, 68, 83, 84, 86] and various *Daphnia* species (e.g. refs 55, 140). Among these cladoceran groups, the genus *Daphnia* has received the most attention. Several reasons account for this imbalance. Most *Daphnia* species are easy to rear in the laboratory (parthenogenetic reproduction), and stock cultures (clones) can be maintained for many years. Furthermore, ecological as well as population dynamic aspects of *Daphnia* species have been studied extensively, compared with other cladoceran groups[43, 49, 87, 126]. Using only morphological traits species exhibiting large intraspecific phenotypic variation are difficult to investigate with regard to interspecific hybridization (e.g. ref. 86). Additional techniques, such as the application of genetic markers (e.g. allozymes) which reveal species and hybrid specific patterns, have proven to be extremely useful in identifying hybrid and parental taxa[55]. Since allozyme electrophoresis requires a certain amount of enzyme per individual, not all species can be subjected to this kind of analysis.

However, studies of *Simocephalus*[50] and *Pleuroxus*[108] have revealed evidence for interspecific hybridization. One interesting phenomenon within the *Simocephalus* species complex is that hybrids are formed not only between closely related species (*Simocephalus congener* and *S. exspinosus*), but also between rather distantly related species as well (*S. vetulus* and *S. serrulatus*[50]). The authors indicate that factors other than genetic distance between taxa (such as the frequency of co-occurrence, and the timing of sexual reproduction among species) may account for these observations.

Studies on chydorids have demonstrated that interspecific hybridization might be rare or non-existent in nature[108]. Laboratory crosses between *Pleuroxus procurvus* and *P. denticulatus* only reproduce parthenogenetically. Since these species need sexual reproduction to form resting eggs (otherwise they could not survive cold periods) it is unlikely that hybrid clones can persist for longer than one season.

Paleolimnological and morphological investigations of various *Bosmina* species indicate introgressive hybridization[68, 83, 84, 86]. During the late-glacial and late Holocene in Lake Constance (southern Germany), a shift of species composition occurred. *Bosmina coregoni* invaded the lake, which was dominated originally by *B. longispina*. After the invasion, both species coexisted with several intermediate forms (hybrids), and later the parental forms disappeared and the population consisted mostly of hybrids[68]. In contrast, across North America no interspecific hybrids of various *Bosmina* species have been detected[26]. Although no $F_1$ hybrids have been found, some populations showed heterozygote excess and *Bosmina coregoni* species exhibited four unique alleles which might have been introduced via hybridization and subsequent introgression with other bosminid taxa[27].

Over the past decade eight species-hybrid complexes have been described among *Daphnia* species (table 1; refs 55, 59, 121, 124, 140). Hybrids between *D. cephalata* and *D. carinata* co-occur with their parental species in ponds throughout eastern Australia[55]. Although the hybrids are capable of sexual reproduction (laboratory breeding studies), no backcrosses or $F_2$ hybrids have been found in nature. This suggests fitness differences between $F_1$ hybrids and parentals versus backcrosses and $F_2$ hybrids, or differential timing of sexual reproduction.

Among obligatory asexual clones of *D. pulex*, two groups have been distinguished on the basis of ecological prevalence and genetic markers. These have been named 'urban' and 'forest' clones[56]. The latter turn out to be hybrids between *D. pulex* and *D. pulicaria*, which have been found across western Canada[23, 56, 59]. These hybrids are thought to be $F_1$ hybrids (based on allozyme analyses) which reproduce asexually (obligate parthenogenesis). Although species and hybrid distributions and variation in breeding systems in the *D. pulex* group are well studied, very little is known about the maintenance of hybridization or the evolutionary consequences of hybridization. However, relatively high genetic divergence between parental species suggests very little (if any) introgression. In concordance with parental species, hybrids showed the highest genotypic diversity in western Canada; in this area species and hybrids frequently co-occur. Thus, hybridization seems to be more frequent in western Canada, and genotypic characteristics imply that hybrids are produced locally[59].

Several species complexes are known in the *D. longispina* group (table 1). Two complexes in North America (*D. galeata galeata/galeata mendotae* and *D. galeata mendotae/rosea*) and four in Europe (*D. cucullata/hyalina*, *D. galeata/hyalina*, *D. cucullata/galeata*, and *D. galeata/longispina*) have been studied. A comparison of *D. galeata mendotae* allozyme genotypes in North America and *D. galeata galeata* genotypes in Europe has revealed a recent introduction of the European sub-species to North America, including subsequent hybridization with *D. galeata mendotae*[124]. Within the hybrid group, several multi-locus genotypes have been found, thus indicating multiple hybridization

Table 1. List of publications concerning population genetic or ecological aspects of interspecific hybridization among *Daphnia* species complexes.

| Species complex | Publications |
|---|---|
| *D. cephalata/carinata* | Hebert[55] |
| *D. pulex/pulicaria* | Hebert et al.[56], Hebert et al.[59] |
| *D. cucullata/hyalina* | Flößner and Kraus[38], Wolf and Mort[140], Gießler[44], Wolf[137], Flößner[37], Müller[96], Schwenk[105], Streit et al.[119] |
| *D. galeata/hyalina* | Flößner & Kraus[38], Wolf and Mort[140], Gießler[44], Wolf[137], Weider and Wolf[132], Wolf and Weider[141], Weider and Stich[131], Flößner[37], Müller[96], Schwenk[105], Spaak and Hoekstra[111], Weider[130], Streit et al.[119] |
| *D. cucullata/galeata* | Flößner and Kraus[38], Wolf and Mort[140], Gießler[44], Wolf[137], Hebert et al.[58], Weider and Wolf[132], Flößner[37], Müller[96], Schwenk[105], Müller and Seitz[97,98], Spaak and Hoekstra[112], Boersma[16], Streit et al.[119] |
| *D. galeata/longispina* | Hebert et al.[58] |
| *D. galeata/galeata mendotae* | Taylor and Hebert[124] |
| *D. rosea/galeata mendotae* | Taylor and Hebert[121-123] |

events, or sexual reproduction of hybrids. Although the $F_1$ hybrids seem to be sexually fertile, only a few backcross or $F_2$ genotypes have been found (using species-specific allozyme markers). This pattern indicates that recombinant genotypes are probably less fit than $F_1$ hybrids and parentals. *D. galeata mendotae* also hybridizes with *D. rosea*, although both species have different habitat preferences[121]. *D. galeata mendotae* is dominant in large lakes, while *D. rosea* is dominant in small lakes and permanent ponds (fish free). Hybrids occurred in lakes of intermediate size together with either *D. galeata mendotae* or *D. rosea*. Hybrid populations are characterized by low genetic variation, but can dominate local populations numerically. Hybrids were temporarily dominant and clones have been found to persist over several years, which suggests higher relative fitness of these hybrids when compared with parentals and also suggests the potential origin of dominant hybrid clones via a few hybridization events[121,123].

Although interspecific hybridization among species of the *D. galeata/hyalina/cucullata* complex has been investigated with respect to ecological differentiation and population genetic patterns, the systematics of the group is still unresolved[44,58,96,98,105,110,111,130-132,136-138,140]. However, the application of allozyme electrophoresis in concert with morphological investigations revealed that *D. galeata*, *D. hyalina*, *D. cucullata* and their interspecific hybrids occur syntopically in various European lakes. Parental species and their hybrids differ in ecological characteristics and patterns of seasonal abundance[110,130,137]. Recombinant genotypes, such as $F_2$ hybrids or backcrosses, seem to be rare, and introgression has been suspected for only one population[110].

Since natural interspecific hybridization seems to occur only among some cladoceran groups (e.g. *Daphnia* and *Bosmina*), several hypotheses have been proposed to account for this pattern. One explanation for the limited extent of hybridization among chydorids and littoral zone daphnids might be short-term survival of hybrids and behavioral specialization (e.g. different habitat preferences) of species[50]. If hybrids reproduce only asexually, and have to cope with rapid changes in environmental conditions (including droughts), which require the production of resting eggs, then the success of clonal hybrid lineages will be minimized. This is of course only true for species which require sexual reproduction to produce resting eggs. In large temperate lakes, zooplankton species do not face extreme environmental perturbations, such as droughts and strong temperature fluctuations. Thus hybrids, once produced, may persist as clonal lineages via asexual reproduction. Although it seems that interspecific hybridization among large-lake species (e.g. genus *Daphnia*) is more common than among temperate pond species, information about interspecific hybridization is still insufficient to draw general conclusions. However, environmental conditions might influence the likelihood of hybridization or the persistence of hybrids, but hybridization per se is facilitated by genetic and historic factors (interspecific cross-fertilization, mode of speciation; e.g. ref. 52).

Hebert[55] suggested that the incidence of interspecific hybridization and temporarily high abundances of hybrids among *Daphnia* and rotifers might be due to their ability to reproduce parthenogenetically. Parthenogenetic reproduction certainly facilitates the occasionally higher abundances of hybrids, compared with parental species. But the occurrence of interspecific hybridization among *Daphnia* and rotifer species is most likely based on semi-permeable reproductive barriers which allow interspecific crosses[105,122]. However, the phenomenon of interspecific hybridization among parthenogenetic animals has to be examined, first for the occurrence of hybridization per se, and second, for the ecological success and persistence of hybrid clones. Hence, in addition to the 'classical' evolutionary questions with regard to interspecific hybridization (mentioned above), other factors such as the consequences of asexual reproduction and the 'evolutionary age' of hybrid clones have to be considered.

Table 2. Diagnostic loci used to discriminate between *Daphnia* species and hybrids[130, 140].

| Taxon | Loci and alleles | | | | | | |
|---|---|---|---|---|---|---|---|
| | sAAT | AO | PGI | PGM | MDH | PEP | GPDH |
| *D. galeata* | f | f | m, f | s, m, f, f$^{+}$ | s, f | f, m | f |
| *D. hyalina* | s | s | m | m, f, f$^{+}$, f$^{++}$ | f, f$^{+}$ | s | s |
| *D. cucullata* | s$^{-}$ | f | s$^{-}$, s, m, f | s, m | s, f, f$^{+}$ | - | - |

Alleles reflect the relative anodal migration distances as follows: f$^{++}$: super fast; f$^{+}$: very fast; f: fast; m: medium; s: slow; s$^{-}$: very slow. (sAAT: aspartate aminotransferase; EC 2.6.1.1; AO: aldehyde oxidase; EC 1.2.3.1; PGI: phosphoglucose isomerase; EC 5.3.1.9; PGM: phosphoglucomutase; EC 2.7.5.1; MDH: malate dehydrogenase; EC 1.1.1.40; PEP: peptidase; EC 3.4.11; GPDH: glycerol-3-phosphate dehydrogenase; EC 1.1.1.8).

## A case study: the *Daphnia longispina* species complex

### Biogeographic patterns and population structure of species complexes

*Daphnia* hybrids (*D. galeata x hyalina, D. cucullata x galeata* and *D. cucullata x hyalina*) have been found at several locations across Europe. Interspecific hybridization has been discovered using species-specific morphological characters and allozyme markers (fixed alleles). $F_1$ hybrids are characterized through intermediate morphology (compared with parentals) and heterozygous genotypes at marker loci (table 2; ref. 140). In the following the term hybrid is used in the sense of mixed ancestry, therefore the group of hybrids may comprise $F_1$ hybrids, $F_2$ hybrids and backcross genotypes. Nearly all *Daphnia* hybrids have been detected in lake areas in southern (Bavaria), northern (Plön), and western Germany (Eifel), as well as The Netherlands (refs 44, 98, 112, 140; Schwenk, unpubl. data). In addition, *D. cucullata x galeata* and *D. galeata x longispina* hybrids are present in several lakes in Bohemia, Czech Republic[58]. This pattern indicates that hybrids occur across central Europe in a rather patchy distribution. Thus, either *Daphnia* species form broad hybrid zones, or species and hybrid distributions primarily reflect the patchy structure of the environment. A similar pattern was found for hybrids of *D. galeata mendotae* and *D. rosea*. They also occur across a relatively large geographic area, which ranges from lakes in northern Indiana, USA, to the province of Ontario, Canada and into New England[121, 123]. The geographically widespread hybridization among these species complexes is a rare phenomenon when compared with other animal species, which typically form rather narrow hybrid zones[11].

Although species of the *D. galeata/hyalina/cucullata* complex have been well studied in regard to ecological preferences, the results could be biased due to uncertainties of species and hybrid discrimination. However, it seems that *D. hyalina* prefers oligotrophic, *D. galeata* meso- to eutrophic and *D. cucullata* eutrophic lakes[36]. Since this ecological discrimination is rather crude, and lake characteristics vary considerably over time, no significant correlation of trophic states of habitats with species occurrence seems feasible[130]. Interestingly, habitat features such as altitude, mean lake depth or lake surface area seem to be better predictors of species occurrence. *D. hyalina* is the only species that occurs in alpine lakes above 1000 m altitude[36], and is not found outside formerly glaciated areas and glaciated areas of the Tatras, Pyrenees and the Balkans[72]. In North America, *D. galeata mendotae* appears to occur in larger lakes than *D. rosea*[121].

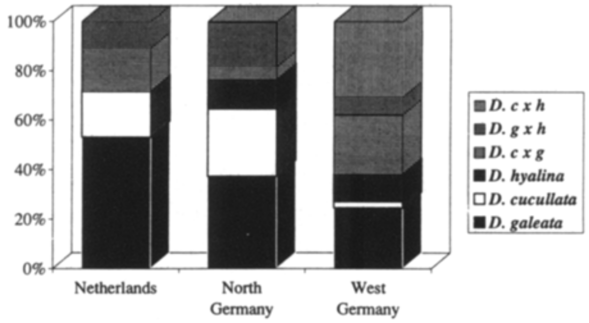

Figure 2. Species and hybrid abundances of three species complexes (*D. galeata/hyalina/cucullata*) among three lake districts in Europe. Netherlands: 30 populations (Schwenk, unpubl. data), North Germany: 14 populations of the Plön lake district[140], West Germany: four lakes from the Eifel and near Mainz[96].

For three areas in northern and western Germany[96, 140] and The Netherlands (Schwenk, unpubl. data), different patterns of species and hybrid abundances have been found (fig. 2). *D. cucullata x galeata* hybrids occur in higher frequencies in lakes of western Germany and The Netherlands than in lakes of northern Germany. *D. cucullata x hyalina* is very abundant in western Germany, but nearly absent in the two other regions. *D. galeata x hyalina* hybrids are present in all three regions, although no *D. hyalina* populations have been found in The Netherlands. In general, hybrids within the *D. galeata/hyalina/cucullata* complex and the *D. galeata mendotae/rosea* complex are found in relatively high frequencies (up to 44%) across large geographic ranges (fig. 3). Although *D. galeata mendotae* hybrids are widespread in North America, the frequencies of hybrids are greatly reduced outside the Indiana-Michigan lake district. This pattern might be related to the pond habitat preference of *D. rosea* outside this area (D. Taylor, pers. commun.).

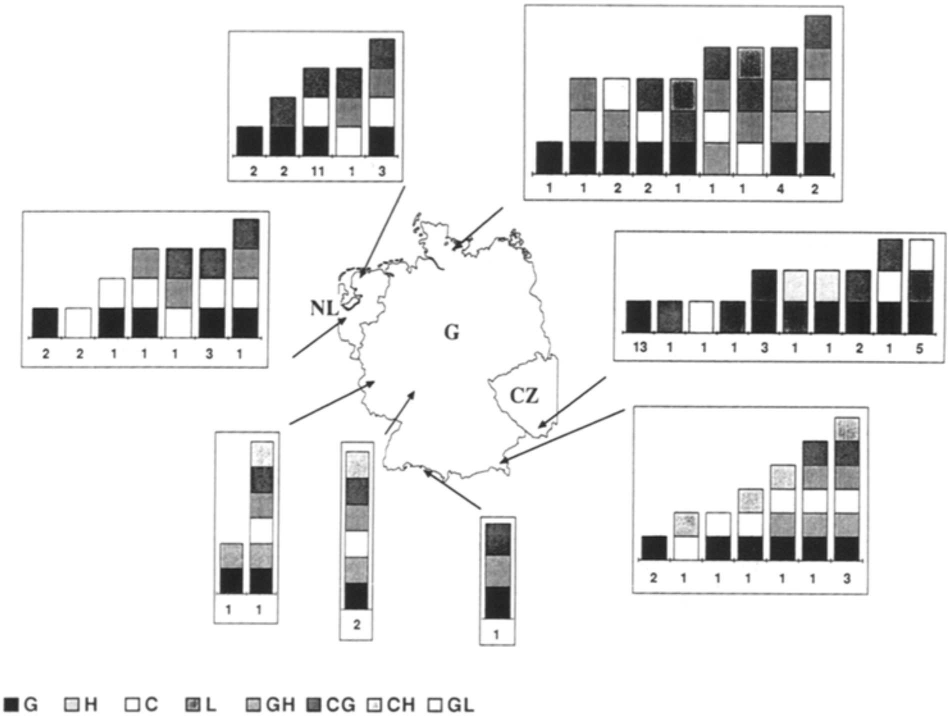

Figure 3. The distribution of four *Daphnia* species (*D. galeata*, *D. hyalina*, *D. cucullata* and *D. longispina*) and their hybrids (*D. galeata x hyalina*, *D. cucullata x galeata*, *D. cucullata x hyalina* and *D. galeata x longispina*) in The Netherlands (NL), Germany (G) and the Czech Republic (CZ). Each bar represents a combination of taxa in a certain lake and the numbers below each bar give the number of lakes of a certain combination of taxa.

To examine the composition of populations, we classified each lake into one of five categories: **A**, only one of the parental species present; **S**, all three taxa occur together (syntopy); **P**, both parental species without hybrids; **H**, only hybrids; or **H + P**, hybrids together with only one of the parental species (fig. 4). This analysis is based on data from 102 lakes across Europe and 22 lakes in North America (refs 44, 58, 96, 121, 140; Schwenk, unpubl. data). Within the *D. cucullata*/*galeata* complex ~ 30% of the populations were composed of both parental species and hybrids (syntopic), whereas the *D. galeata*/*hyalina* and the *D. hyalina*/*cucullata* complexes showed lower values. Since not all lakes were sampled more than once per year, which may cause a bias in the estimation of the number of taxa present, we expect the proportion of syntopic populations to be even higher. In all three complexes however, the majority of populations is composed of only one parental species, *D. galeata* within the *D. cucullata*/*galeata* and *D. galeata*/*hyalina* complexes, and *D. cucullata* within the *D. hyalina*/*cucullata* complexes. Whether these patterns are caused by ecological or historical factors remains an open question. However, evidence for size-selective predation and the influence of food quality and quantity on species composition has been adduced[112,130]. Interestingly, in some populations no hybrids have been detected although both parental species are present. This implies that species/clones are reproductively isolated either by asynchronous sexual reproduction of parentals or by other intrinsic barriers (such as genomic incompatibility) to gene flow[110,137]. Compared with the *D. galeata*/*hyalina*/*cucullata* complex, the *D. galeata mendotae*/*rosea* complex shows an entirely different pattern (fig. 4). No syntopic populations (S), or populations with both parental species (P) have been found, but similar proportions of populations composed of either only hybrids, hybrids together with one of the parental species and only *D. galeata mendotae* exist. Taylor and Hebert[121] suggested that clear ecological preferences of *D. galeata mendotae* for larger lakes and *D. rosea* for smaller lakes could explain this pattern. In addition, more populations are composed solely of hybrids than of only one parental species. All these patterns taken together indicate that hybrids can not only cope with both parental habitat types, but that hybrids seem to successfully exploit an intermediate ecological niche. Although hardly any syntopic populations of both parentals and hybrids occur, the proportion of hybrid populations ( > 70%; fig. 4) and the local abundance of hybrids ( > 40%) is very high. These proportions are smaller for populations of the *D. galeata*/*hyalina*/*cucullata* complex, (10–45% and 12–26%, respectively), although syntopic populations are frequently found (30%). This pattern might be explained by assuming that species of the *D. galeata mendotae*/*rosea* complex are spatially separated through their ecological differentiation, whereas species of the *D. galeata*/*hyalina*/*cucullata* complex are separated primar-

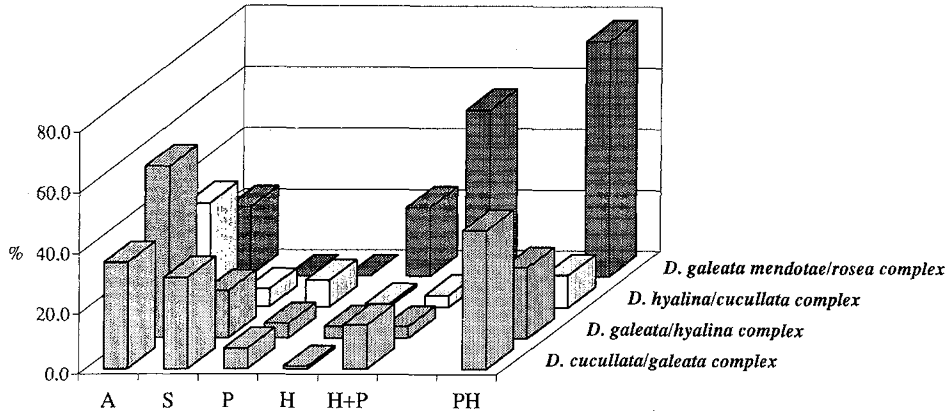

Figure 4. Species and hybrid compositions of 124 populations of four hybrid complexes (*D. galeata/hyalina*, *D. galeata/cucullata*, *D. hyalina/cucullata* and *D. galeata mendotae/rosea* complex) across Europe and North America divided into five classes of populations. (**A**: only one parental species per lake, **S**: hybrids occur together with both parental species (syntopy), **P**: both parental species without hybrids, **H**: only hybrids, **H + P**: hybrids together with only one of the parental species). Bars on the far right show the percentage of populations in which hybrids were detected (**PH**).

ily by various levels of reproductive isolation (seasonally asynchronous sexual reproduction or intrinsic barriers to gene flow).

## Maintenance of hybrid lineages and fitness comparisons

One of the most effective ways to reveal the potential evolutionary significance of hybridization and introgression is to measure the relative fitness of different hybrid and parental genotypes[5]. This might be especially illuminating for cyclic parthenogens like *Daphnia*, which can circumvent the disadvantage of reduced sexual fertility. In principle, hybrid genotypes which bear combinations of advantageous traits can be maintained indefinitely in a population through asexual reproduction. Thus, the comparison of intrinsic rates of increase (*r*) of parental and hybrid genotypes can be used as a measurement of fitness. Although *r* represents only one component of fitness and a definition of fitness among sexually-asexually reproducing taxa is not straightforward, the estimation of *r* and other life history characteristics reveal the capability of clones to respond to environmental fluctuations (ecological success, environmental sensitivity). In addition, size-related traits were taken into account, since both invertebrate and vertebrate predation on *Daphnia* is size-selective[142].

Compared with genetic studies, fitness differences of *Daphnia* hybrids and their parental species have been less investigated. The available studies are restricted to the *D. galeata/hyalina/cucullata* complex[16,112,130,132,141]. In these studies, life history characteristics of hybrids and parental species were compared with respect to food level, temperature and differences in vertical distribution[97,131].

Hybrids and parentals were found to be significantly different in most life history characters. The intrinsic rate of increase (*r*) of *D. galeata x hyalina* was significantly higher compared with the parental species at low temperature (14 °C), while at high temperature (20 °C) the parental species performed better[141]. In their extended study, Weider and Wolf[132] showed that *D. cucullata x galeata* hybrids have a *r* that differs significantly from *D. cucullata* but does not differ significantly from *D. galeata*. The size of *Daphnia* hybrids (at birth and maturity) is in most cases intermediate to the parental species; however, only one or two clones per taxon were used.

In a study on the niche breadth of *D. galeata x hyalina* and its parental species using five clones of each taxon raised at four food levels ranging from 0.2 to 2 μg C $ml^{-1}$, Weider[130] found significant taxon *x* food level interactions for *r* as well as for size-related traits. At extreme food levels (0.2 and 2 μg C $ml^{-1}$), the *r* of hybrids did not differ significantly from *D. galeata* but both had a significantly higher *r* when compared with *D. hyalina*. Additionally, hybrid clones are characterized by intermediate body size compared with parental species. Differences in niche breadth could not be shown between the hybrids and parentals; only *D. galeata* and *D. hyalina* differed significantly in this respect. A similar pattern has been discovered for *D. cucullata*, *D. galeata* and their hybrids; both species compete with the hybrids (food), but not among each other, which suggests limited niche overlap of species[16].

It might be that fluctuating environmental conditions, e.g. for temperature, food levels and predation regimes, create conditions under which hybrids temporarily have higher fitness than the parental species. This hypothesis is supported by the temporal distribution of syntopically occurring hybrids and parentals; in certain periods of the year hybrids dominate (relative abundance) the species complexes[111,137].

The hypothesis that *Daphnia* hybrids are maintained through temporarily higher fitness (temporal hybrid superiority model), was tested by Spaak and Hoek-

Table 3. Ecological characteristics of *D. galeata*, *D. cucullata* and *D. cucullata x galeata*.

| Taxon | Predation risk fish | Predation risk invertebrate | $r$ | Competition (interspecific) |
|---|---|---|---|---|
| *D. galeata* | high | low | high | low |
| *D. cucullata* | low | high | low | low |
| *D. cucullata x galeata* | intermediate | intermediate | high | high |

stra[112] with *D. galeata*, *D. cucullata* and hybrid clones from Lake Tjeukemeer, The Netherlands. In the laboratory, mean $r$ of the hybrids did not differ significantly from *D. galeata*, but was significantly higher than in *D. cucullata*. Furthermore, life history comparisons of hybrids and parentals demonstrated that $r$ values for hybrids are higher at high food conditions than those of parentals[16]. Because the hybrids are significantly smaller than the larger parental *D. galeata* and predation by fish is positively size-selective, they will have an advantage in the field. Fish predation on *Daphnia* is very high in this lake[77,127]. Boersma et al.[17] showed that smelt is a size-selective predator on *Daphnia*: in June, smelt selected for smaller *Daphnia*, and in the rest of the year for larger daphnids, especially individuals $>1$ mm body length. In addition, during certain periods of the year size-dependent death rates reach 10% $d^{-1}$ for daphnids around 1 mm body length to $>60\%$ $d^{-1}$ for daphnids around 1.6 mm body length[128]. These data show that the smaller size at maturity for *D. cucullata* and the hybrids can be selectively advantageous.

The observed differences in size and fecundity between the taxa may explain why *D. galeata* and the hybrids dominate the *Daphnia* assemblage in Lake Tjeukemeer early in the season (higher $r$, low predation risk, high food concentrations), and *D. cucullata* in the summer with high fish predation (lower vulnerability to fish predation, lower food concentrations). For example, at the end of May 1989 both *D. galeata* and the hybrids reached peak densities (65 ind./l), which lasted for about one week for *D. galeata*, but the hybrid population did not collapse until August[110]. The dominance of *D. cucullata* afterwards can thus be explained by size-related differences in vulnerability to visual predators (fishes). In a three-year study of the population dynamics of the *D. cucullata/galeata* species complex in Lake Tjeukemeer, Spaak[110] provided evidence to support the temporal hybrid superiority hypothesis. Deviations from the mean instantaneous rate of increase on a certain date ($r_r$) showed a positive relationship with fish predation for *D. cucullata*, a negative one for *D. galeata*, and no relationship for the hybrids. This suggests a higher influence of fish predation on the $r$ of the larger *D. galeata* compared with the smaller hybrids and *D. cucullata*. Periods with a positive $r_r$ were found to be shorter for parental species than for the hybrids.

Temporal studies on the occurrence of hybrids and parental species in lakes have shown that the relative abundances of these taxa differ[110,131,137]. In Lake Constance, *D. galeata* was more abundant from May to September, compared with *D. hyalina*, while the proportion of hybrids varied between 20 and 40% during the whole year[131]. A three-year study in Lake Tjeukemeer showed that large among-year differences in species composition can occur[110]. For example, in the summer of 1990 the daphnid population was composed of 30% *D. cucullata x galeata* hybrids but in 1991 they decreased to less than 10%. These results suggest that the relative abundance of taxa is determined by the interaction of life history features and environmental factors (e.g. food, temperature and predation).

Besides variation in life histories, differences in the vertical distribution of *Daphnia* hybrids and parental species can account for niche segregation. Weider and Stich[131] found that *D. galeata* was the dominant taxon in the upper 20 m of Lake Constance during summer and early autumn, and *D. hyalina* and the hybrids were most abundant below 30 m. A comparable pattern was found for *D. galeata* and the hybrid *D. cucullata x galeata* in Lake Neuhofener Altrhein (Germany) by Müller and Seitz[97]. Day depth of *D. galeata* was confined to the border between the meta- and hypolimnion, whereas the smaller hybrids showed a more heterogeneous distribution. This result might reflect the lower predation risk for the smaller hybrids compared with the larger *D. galeata*.

Characteristics of hybrids, such as $r$, interspecific competition and vulnerability to predation (fish and invertebrates) show significant differences to parental species (table 3). This pattern suggests niche segregation, and might explain the temporarily high abundance (ecological success) of hybrid clones. If hybrids reproduce preferentially via parthenogenesis, hybrid clones can be maintained because of their ecological differentiation from parental species. The mode of reproduction in combination with the variation of environmental factors seems to be the essential presupposition for the ecological success of hybrids; hybrids might be characterized by high clonal reproductive success but low sexual fertility.

## Genetic and evolutionary consequences of interspecific hybridization

Consequences of natural hybridization may comprise: 1) merging of populations, 2) reinforcement of reproductive barriers, 3) introgression[4], 4) speciation[19], 5) hybrid swarms persist in areas of disturbance[2] or 6) hybrid populations form 'sinks' for pest organisms[134]. The origin and dynamics of the hybridization process is dependent on levels of reproductive isolation, ecological differentiation between hybridizing populations and factors such as genetic drift, migration, assortative mating and selection on hybrids[5]. Some of these issues have been investigated using the *D. galeata/hyalina/cucullata* and the *D. galeata mendotae/rosea* complexes[44,105,112,119,121–123]. In the following we discuss four aspects: 1) the origin of hybrid lineages, 2) mitochondrial and nuclear DNA introgression, 3) the directionality of hybridization and 4) phylogenetic relationships.

**The origin of hybrid lineages.** In the context of daphnid life cycles/reproduction, two important questions arise: 1) How did hybrids originate, and 2) Are they maintained through ameiotic parthenogenesis as 'evolutionary old' asexual lineages? Population genetic investigations of hybrid populations of the *D. galeata/hyalina/cucullata* complex have revealed more multi-locus genotypes and higher values of clonal diversity compared with hybrids among the *D. galeata mendotae/rosea* complex[110,121]. For example, 32 multi-locus genotypes of *D. cucullata x galeata* hybrids have been found using only three polymorphic loci[110], whereas Taylor and Hebert[121] found between one and five multi-locus genotypes of *D. galeata mendotae x rosea* hybrids using nine allozyme loci. It appears that hybrids are more frequently produced among species within the *D. galeata/hyalina/cucullata* complex, than within the *D. galeata mendotae/rosea* complex, which might be explained by the distribution of parental species. As shown in figure 4, only species of the *D. galeata/hyalina/cucullata* complex frequently occur syntopically, whereas species of the *D. galeata mendotae/rosea* complex coexist infrequently. However, the possibility remains that reproductive isolation, strong selection (such as size-selective predation), or different levels of sexual reproduction among species/clones may be responsible for the number of genotypes detected.

Since *Daphnia* species are able to reproduce parthenogenetically, and in addition obligately parthenogenetic clones occur (e.g. in the *D. pulex* group), in principle hybrids of the *D. longispina* group could be maintained as clonal lineages. One approach to reveal whether hybrids constitute distinct clonal lineages is to compare mitochondrial DNA sequences of hybrid clones and clones of their maternal species. Within the *D. galeata/hyalina/cucullata* complex nucleotide divergence of hybrids and maternal species is virtually identical to divergence among clones within species[105]. In addition, genotypic frequencies of hybrids not only vary over seasons, but also between years[112]. These findings suggest that hybrids do not form old independent asexual lineages, but that they are produced continuously. If hybridization occurs frequently and sexual reproduction between parental species and hybrids occurs occasionally[58,121], then a further question is raised: does genetic material via repeated backcrossing of recombinants cross species boundaries, i.e. does introgression occur?

**Mitochondrial and nuclear DNA introgression.** Among *Daphnia* species the occurrence of introgression has been investigated using morphological characters[84,85]. Since this approach is based on morphological traits whose genetic bases are unknown, additional markers such as allozymes, nuclear and mitochondrial DNA have recently been applied[33,105,110,122]. *Daphnia* backcrosses have been detected occasionally at high frequencies[44,58,96,121], and UPGMA cluster analyses of hybrids and parentals have revealed on average a closer relationship between the hybrid and one of the parental species[96]. An UPGMA analysis of more than 5000 individuals over a period of three years showed that *D. cucullata x galeata* hybrids are more closely related to *D. galeata* (difference $< 0.04$), than to *D. cucullata* (difference 0.30[110]). A similar investigation which applied numerous nuclear DNA markers (*r*andom *a*mplified *p*olymorphic *D*NA; RAPD analysis) has revealed a similar pattern[33]. Evidence for backcrossed genotypes has been found in several species complexes. For example, the frequency of backcrosses per lake ranges from <9% in northern Germany[140] to <10% to 91% in Indiana[121] to <11% in southern Germany (S. Gießler, pers. commun.) and <5 to 85% in Bohemia[58]. Since these circumstances define the necessary conditions for gene flow between species, introgression should be expected.

For both the *D. galeata mendotae/rosea* and the *D. cucullata/galeata* complex, evidence of nuclear DNA introgression has been reported[110,122]. In neighboringly sympatric populations of *D. galeata mendotae* and *D. rosea*, alleles have been detected which are absent or rare in allopatric reference populations. This pattern has been interpreted as introgression of nuclear alleles, mainly from *D. rosea* to *D. galeata mendotae*. A second example of introgression revealed gene flow from *D. cucullata* to *D. galeata*[110]. Since it is difficult to discriminate between pattern of introgression and maintained polymorphisms from a common ancestor, or mutational events, comparisons of independent markers such as morphological characters, nuclear and mitochondrial DNA are needed to falsify the hypothesis of introgression[5,51].

Mitochondrial DNA introgression has been investigated using species-specific mitochondrial DNA markers within the *D. galeata/hyalina/cucullata* and the *D. galeata mendotae/rosea* complexes. In neither case could mitochondrial introgression be detected whereas nuclear introgression has been discovered[105,122]. However, it is possible that the sample size was too low to detect small amounts of mitochondrial gene flow, since it has been found that among animals mitochondrial DNA crosses species boundaries more easily than nuclear DNA[7,35]. An additional explanation could be the following scenario: hybrids within the *D. cucullata/galeata* complex are formed through crosses between *D. cucullata* females and *D. galeata* males. Thus, $F_1$ hybrids possess mitochondrial DNA of *D. cucullata* and on average 50% of each parental nuclear genome. Hybrid females of the *D. galeata/hyalina* complex frequently produce empty ephippia, which indicates that hybrid females probably contribute very few sexually produced offspring[22]. If *D. cucullata x galeata* hybrid males cross with females of *D. galeata* repeatedly, introgressants result which exhibit mitochondrial DNA from *D. galeata* and nuclear DNA mainly from *D. galeata* but also, to a lesser extent, nuclear DNA from *D. cucullata*. Thus, such a process would result in limited nuclear DNA introgression, but no mtDNA introgression. In addition, members of the *D. longispina* complex exhibit relatively high values for nucleotide divergence of mtDNA, although species are considered to be closely related, and interspecific hybridization and probably backcrossing occurs[105]. This pattern might be a result of the differential contributions of hybrid females and males to backcrossing.

**The directionality of hybridization.** Another aspect of interspecific crossing concerns the directionality of hybridization. Are hybrids always formed by females of one parental species (unidirectional hybridization), or are reciprocal crosses possible (bidirectional hybridization)? Several studies have demonstrated that directionality is influenced by either 1) abundances of parental species[9,31], 2) asymmetric interspecific mate choice or reproductive isolation[6,18,30,54,76,99], or 3) differential viability[62,93].

*D. galeata mendotae/rosea* hybrids were found to exhibit mitochondrial DNA from both parental species[122]. This pattern was found to be frequency dependent; in most cases only the dominant parental species passed on its mitochondrial DNA, which could be attributed to environmental induction of sexual reproduction in *Daphnia*. Under this hypothesis, the production of males and sexual females differs slightly in quality and quantity of necessary stimuli. Sexual females can be induced through changes in photoperiod, low food concentration and crowding, whereas males are induced by changes in photoperiod, or a chemical cue which is produced during crowding of daphnids[67,78]. Furthermore, this chemical cue has been used successfully to induce males in different species, thus it does not seem to be species-specific[67]. Given these conditions, Taylor and Hebert[122] proposed the following scenario: Sexual *D. galeata mendotae* females are produced because of the high density of *D. galeata mendotae* clones, whereas due to low density of *D. rosea* clones only *D. rosea* males are formed (because of chemical induction by *D. galeata mendotae*), or vice versa. Thus, the dominant species in such a system would determine the directionality of hybridization.

A different pattern of directionality of hybridization has been revealed among species of the *D. galeata/hyalina/cucullata* complex. Based on limited mtDNA data it appears that unidirectional hybridization is prevalent, although in some lakes bidirectional hybridization has been detected (ref. 105; Schwenk, unpubl. results). Since in Europe, species and hybrids of the *D. galeata/hyalina/cucullata* complex occur in syntopy, and also in similar proportions, the null hypotheses would be that hybrids exhibit mitochondrial DNA of both parentals in similar frequencies. But the data generated so far seem to reject this hypothesis because significantly more *D. cucullata x galeata* hybrids exhibit mitochondrial DNA of *D. cucullata*; similar results hold for the *D. galeata/hyalina* complex (Schwenk, unpubl. results). One possible explanation might be that sexual females and males of both parental species rarely co-occur at the same time during the year[110,137]. These observations suggest that either temporal differentiation of sexual reproduction or asymmetrical reproductive isolation contribute to nonrandom mating among parental species.

**Phylogenetic relationships.** Another consequence of interspecific hybridization and introgression is the reticulation of phylogenetic relationships, rather than hierarchical patterns of lineage diversification. Occasional horizontal gene flow between lineages might cause estimated phylogenies based on morphological, nuclear DNA, and cytoplasmic DNA data to be discordant (e.g. refs 5, 28, 75, 79, 92, 109).

Due to phenotypic plasticity, cyclomorphosis and interspecific hybridization of species in the *D. longispina* group, morphological investigations used to reconstruct phylogenetic relationships have been highly controversial[14,37,38,41,72,85]. Phenetic and cladistic analyses using 42 morphological traits among 43 *Daphnia* species even failed to establish the existence of a *D. longispina* group[14]. Alternatively, Hrbáček[72] grouped *D. galeata*, *D. hyalina* and *D. cucullata* as well as *Daphnia longispina* and *Daphnia rosea* together as one clade. In addition, Wolf and Mort[140], and Hebert et al.[58] suggested that the occurrence of interspecific hybridization as well as electrophoretic data indicate that these species are closely related. However, more genetic information is needed to evaluate species affinities, since morphological criteria alone are not sufficient to resolve the phylogenetic relationships among closely-related *Daphnia* species. Recent reappraisals of the phenotypic variation in *D. galeata mendotae*, *D. galeata galeata*, *D. hyalina* and *D. cucullata*, using additional genetic markers (allozyme analysis), revealed cryptic species complexes and frequent interspecific hybridization (*D. longispina* complex; refs 121, 140). Thus, taxonomic uncertainties in the past were most likely caused by the production of intermediate forms and several 'recombinations' of species-specific traits. However, within the *D. galeata/hyalina/cucullata* complex coherent phylogenetic relationships have been found using morphological traits, allozyme and nuclear DNA markers (refs 33, 96; S. Gießler, pers. commun.). *D. galeata* and *D. hyalina* seem to be more closely related to each other than either of them is to *D. cucullata*. However, an alternative branching order was suggested based on mitochondrial DNA information[105]. *D. galeata* and *D. cucullata* appear to be more closely related than either of them is to *D. hyalina*. If the tendency of discordance between nuclear and mitochondrial phylogenies is supported by further studies (and lineage sorting can be excluded), we may infer incidences of reticulate evolution in this group.

## Synthesis

Interspecific hybridization seems to be common among *Daphnia* species, rare in *Bosmina* and rare to non-existent among other cladoceran groups (e.g. Chydoridae, Sididae and Holopedidae). Since the ecology and genetics are far better understood for *Daphnia* than for other cladoceran genera, it is possible that the extent of hybridization among cladocerans is underestimated. However, hybridization occurs within the subgenera *Ctenodaphnia* (*Daphnia carinata* complex) and *Daphnia* sensu strictu (*D. pulex* and *D. longispina* group). The latter is characterized by high levels of hybridization (approximately 30% of all species form hybrids), and hybrids are found across broad geographic ranges.

The most distinct characteristics that separate cladoceran, particularly *Daphnia* species complexes from most other animal species complexes investigated so far are: 1) the island-like nature of lake habitats, 2) the occurrence of hybrids and parentals in sympatry and even syntopy across large geographic areas, and 3) a breeding mode (cyclical parthenogenesis) which provides the possibility for hybrids to circumvent any deleterious effects of reduced sexual fertility by relying on asexual reproduction. The first characteristic facilitates local hybridization; hybrids from different lakes vary in genotypic characteristics: they are probably not dispersed, but once they are produced they are able to persist locally via parthenogenetic reproduction[123]. In the *D. galeata mendotae/rosea* complex, only a few hybrid genotypes are found, which reflects either strong selection against certain genotypes or rare hybridization events. Since the parental species are rarely found to coexist syntopically, the latter explanation may hold true. Lakes seem to be markedly different in their ecological settings, and species have clear preferences for certain habitats, thus the island-like character of lakes and low dispersal rates are responsible for a small number of interspecific crosses. However, if intermediate habitats are available ('hybridization of the habitat'; ref. 2), then opportunities for hybridization increase. In other species complexes (*D. galeata/hyalina/cucullata*) the frequency of habitats in which species co-occur is significantly higher, and more hybrid genotypes are found. In addition, variation in genotype frequencies between years[110] and variation of species and hybrid compositions across lake regions[130] suggest that the maintenance of hybrid genotypes largely depends on suitable ecological parameters of lakes, such as predation and food regimes.

Among the *D. galeata/hyalina/cucullata* complex, genotype compositions of hybrid populations are highly diverse and variable between years, but some lakes are known to be dominated by only one hybrid genotype for longer periods[110,111]. These observations raise the question: how are hybrid lineages maintained? To evaluate this issue, we need to investigate fitness characteristics of species and hybrids. Hybrid fitness can be differentiated into two aspects: first, the comparison of fitness values of hybrids and parentals per se (asexual phase of life cycle; e.g. predation risk, *r*), and second the sexual contribution of hybrids to the next generation (sexual phase of life cycle; lifetime reproductive success). With regard to the first issue, empirical data support the view that hybrids could be, at least temporarily, superior to parental species. Once produced hybrids might occupy an ecological niche, either through differential diel vertical migration strategies[97,131], or through a combination of traits which reduce predation risks[112]. However, if hybrids reproduce only parthenogenetically, then their potential for genetic adaptation depends solely on mutation. As a consequence, hybrids could be locally and within an 'ecological time scale' very important, but of minor or no importance on an 'evolutionary time scale'. In theory hybrids are able to follow several evolutionary pathways (fig. 5). They can either persist as a parthenogenetically reproducing clonal lineage (a), become extinct (b), or reproduce sexually with parentals or other hybrid clones (c). Life history characteristics help to elucidate the short-term ecological success and the niche breadth of hybrid clones, whereas the evolutionary significance of interspecific hybridization depends on the (sexual) reproductive success of hybrids ($F_2$ or backcross genotypes).

One way of maintaining advantageous traits, which have become established through successful hybridization, is to switch from cyclical to obligate parthenogenesis. Certain clones of the *D. pulex* group are able to produce resting eggs asexually, hence they evolve independently from their sexual ancestors[57]. In contrast, no obligately parthenogenetic clones have been detected so far in the *D. galeata/hyalina/cucullata* complex, thus hybrid or backcross clones can only maintain their advantageous traits by subsequent parthenogenetic re-

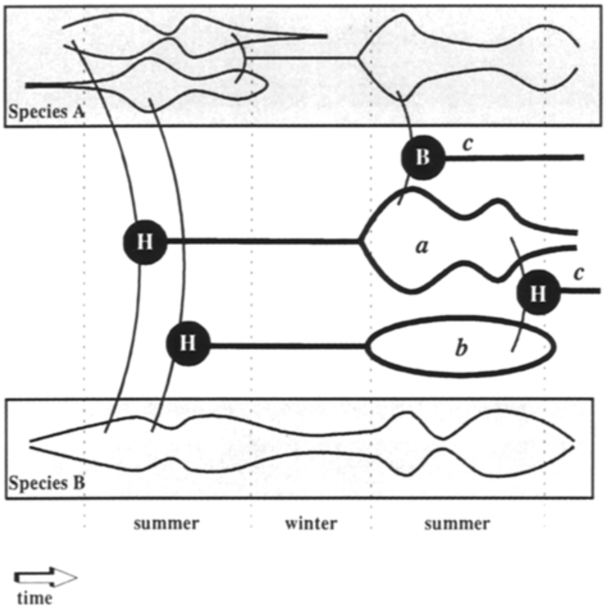

Figure 5. Model of *Daphnia* interspecific hybridization with regard to reproductive modes and clonal abundances. Envelopes (width = abundance) symbolize parthenogenetic reproduction, lines sexual reproduction and subsequent resting egg stages and black circles hybridization (H) and backcross events (B). If hybrids are produced they might persist for one season (or even longer) as resting eggs before they hatch and reproduce asexually via parthenogenesis (a), or they become extinct after a certain time of asexual reproduction (b). If hybrids are able to reproduces sexually, they might form backcrosses or $F_2$ hybrids (c).

production. However, to what extent hybrids are limited in their capability to reproduce sexually because of deleterious effects during meiosis (i.e. hybrid sterility) remains an open question. Evidence of nuclear introgression[110,122] together with the directionality of hybridization and backcrossing[105,122] suggest that probably hybrid males are involved in sexual reproduction. However, data from crossing experiments are needed to reveal the degree of reproductive and genetic isolation of hybrids.

Furthermore, it is of interest to know to what extent competition between species and hybrids is responsible for the fate of a species complex. Recent ecological data on resource competition in *D. galeata*, *D. cucullata* and the *D. cucullata x galeata* hybrids suggest no competition between species, but competition between hybrids and each parental species[16]. In this context hybridization seems disadvantageous for parental species and one could hypothesize a process which reinforces reproductive isolation and decreases the frequency of hybridization[71]. However, competition for food is most likely not the only competitive factor, and barriers to gene exchange could also be derivations of allopatric speciation[70].

Another aspect of interspecific hybridization is the evolutionary history of the interacting species, which provides valuable data for explaining current patterns of hybridization. Most hybrid zones in Europe have been interpreted as secondary contact zones of species which had previously diverged in separated refugia during glacial periods. Other explanations consider environmental disturbances (such as those caused by human activities), as relevant for facilitating hybridization. A third hypothesis assumes differentiation in sympatry according to e.g. environmental gradients which leads to lineage splitting[34]. This scenario includes a stage where reproductive isolation is incomplete and hybrids are still produced. Although many lakes have been subjected to massive changes due to man-made eutrophication and pollution, it seems unlikely that hybridization is caused solely by environmental disturbances. Since interspecific hybridization comprises several *Daphnia* species and occurs on different continents (America, Europe, Australia), other than ecological factors seem to be involved as well. It also seems unlikely that sympatric differentiation occurs, since theoretical studies predict founder effect speciation for *Daphnia* species[88]. Unfortunately, data about species and hybrid biogeography in Europe are very fragmentary and a phylogeny of the whole *D. longispina* group is not available, hence firm conclusions from published data concerning historic (glaciation, speciation) or current processes (dispersal) are hardly possible at present. In general, no hybrid zones as often seen for terrestrial examples seem to exist for *Daphnia* species complexes; hybrids occur in a rather patchy distribution across Europe and North America. Selection against hybrids is reduced, and short-term advantages of hybrids may accrue from ecological factors. Environmental parameters determine the short-term fate of *Daphnia* hybrids, whereas long-term evolutionary consequences may result from interspecific gene flow.

## Future perspectives

Although initial results on cladoceran species complexes have shown ecological and genetic patterns which describe and explain the evolutionary process of interspecific hybridization, some central questions remain. Is interspecific hybridization restricted to *Daphnia* and *Bosmina* species within the group of freshwater cladocerans? Is interspecific hybridization among *Daphnia* and *Bosmina* species an important evolutionary factor, or nothing more than inconsequential evolutionary noise?

Both reproductive and genetic isolation requires further investigation. If one assumes that hybrids rarely reproduce sexually and are incapable of resting egg production via apomixis (which might be true for most of the hybrids within the *D. longispina* complex), we expect that hybrids are able to persist until extinction in their lake of origin since no dispersal is possible. Consequently, a comparison of hybrid and parental species genotypes of various isolated sites should reveal higher levels of genetic divergence between hybrid populations than between species populations. The other extreme would be that hybrids reproduce sexually, which could lead to speciation via hybridization or, more likely, to fusion of parental taxa[20]. However, data on hybrid classes (such as $F_1$, $F_2$ and backcrosses) and induction of sexual females and males of parental species could provide information about the level of species isolation. The study of interspecific hybridization among zooplankton species (cyclic parthenogens) provides two important additional features compared with 'classical' examples among animals. First, it is possible to separate long-term evolutionary and short-term ecological consequences of interspecific hybridization. Although hybrids might exhibit very low sexual fertility, they can nevertheless dominate zooplankton communities due to their capacity for parthenogenetic reproduction. Hybrids within the *D. longispina* group form genetically diverse groups, thus various combinations of parental genes will be subjected to selection. Comparisons of life history characteristics and predation regimes are bound to reveal which parental characters are under hard selection (predation, resource competition). Second, zooplankton species are relatively easy to cultivate in laboratories and have short generation times, which facilitates experiments on life history variation, selection and predation.

Some extremely interesting but difficult questions that need to be addressed are related to the phenomenon of resting egg formation. What is the ecological and evolutionary significance of resting egg pools? And how frequent are dispersal events of *Daphnia* between habitats? Since it is known that hybrids can hatch successfully from ephippia[139], it would be interesting to know the relative frequencies, hatching rates and the maximum duration of diapause. Production of resting eggs can serve as a predator avoidance mechanism[48], or a process which maintains high genetic diversity. Since several lakes have been found in which only hybrids are present, the question arises whether these populations were founded by dispersed ephippia or whether the parental species were displaced by hybrids and went extinct. Two sources of information could be used to evaluate this question. First, genetic analysis of resting eggs from lake sediments could provide a unique record of the population structure in the past. Secondly, estimation of dispersal rates might indicate how often, or how likely species succession occurs in lakes.

Although *Daphnia* species have been studied in regard to ecological and taxonomical characteristics, the lack of biogeographic data is evident. Based on the wealth of morphological data, additional species-specific characteristics are necessary to reveal species affiliations. Molecular markers such as allozymes or mitochondrial DNA have proven to be of enormous help[104,105,119,122]. In particular, the *D. galeata/hyalina/cucullata* complex offers a unique possibility to study the biogeographic aspects of hybridization, since ecological data are available for numerous lakes and species-specific molecular markers (nuclear and mitochondrial DNA) are by now established.

Acknowledgements. Generous and detailed advice from Lawrence Weider and Thomas Städler significantly improved the presentation of these ideas. We thank Jos van Damme, Ramesh Gulati, Riks Laanbroek, Wolf Mooij, and Jacobus Vijverberg for comments on an earlier version of this manuscript. The comments of Derek Taylor and Richard Harrison also substantially improved the manuscript. We appreciated many stimulating discussions and support by Maarten Boersma, Michaela Brehm and Eva Mader. We are grateful to Sabine Gießler, Rita deMelo, Paul Hebert and Derek Taylor for supplying unpublished data.

1 Abbott, R. J., Plant invasions, interspecific hybridization and the evolution of new plant taxa. Trends Ecol. Evol. *7* (1992) 401–405.
2 Anderson, E., Hybridization of the habitat. Evolution *2* (1948) 1–9.
3 Anderson, E., Introgressive hybridization. Biol. Rev. *28* (1953) 280–307.
4 Anderson, E., and Hubricht, L., Hybridization in *Tradescantia*. III. The evidence for introgressive hybridization. Am. J. Bot. *25* (1938) 396–402.
5 Arnold, M. L., Natural hybridization as an evolutionary process. A. Rev. Ecol. Syst. *23* (1992) 237–261.
6 Arntzen, J. W., and Wallis, G. P., Restricted gene flow in a moving hybrid zone of the newts *Triturus cristatus* and *T. marmoratus* in western France. Evolution *45* (1991) 805–826.
7 Aubert, J., and Solignac, M., Experimental evidence for mitochondrial DNA introgression between *Drosophila* species. Evolution *44* (1990) 1272–1282.
8 Avise, J. C., Bermingham, E., Kessler, L. G., and Saunders, N. C., Characterization of mitochondrial DNA variability in a hybrid swarm between subspecies of bluegill sunfish (*Lepomis macrochirus*). Evolution *38* (1984) 931–941.
9 Avise, J. C., and Saunders, N. C., Hybridization and introgression among species of sunfish (*Lepomis*): analysis by mitochondrial DNA and allozyme markers. Genetics *108* (1984) 237–255.
10 Barton, N. H., The fitness of hybrids between two chromosomal races of the grasshopper *Podisma pedestris*. Heredity *45* (1980) 47–59.
11 Barton, N. H., and Hewitt, G. M., Analysis of hybrid zones. A. Rev. Ecol. Syst. *16* (1985) 113–148.
12 Barton, N. H., and Hewitt, G. M., Adaptation, speciation and hybrid zones. Nature *341* (1989) 497–503.
13 Barton, N. H., and Jones, J. S., Mitochondrial DNA: new clues about evolution. Nature *306* (1983) 317–318.
14 Benzie J. A. H., Phenetic and cladistic analyses of the phylogenetic relationships within the genus *Daphnia* worldwide. Hydrobiologia *140* (1986) 105–124.
15 Bert, T. M., and Harrison, R. G., Hybridization in western Atlantic stone crabs (genus *Menippe*): evolutionary history and ecological context influence species interactions. Evolution 42 (1988) 528–544.
16 Boersma, M., On the Seasonal Dynamics of *Daphnia* Species in a Shallow Eutrophic Lake. Ph.D. thesis, University of Amsterdam, The Netherlands 1994.
17 Boersma, M., van Densen, W. L. T., and Vijverberg, J., The effect of predation by smelt (*Osmerus eperlanus*) on *Daphnia hyalina* in a shallow eutrophic lake. Verh. int. Verein. theor. angew. Limnol. *24* (1991) 2438–2442.
18 Bradley, R. D., Baker, R. J., and Davis, S. K., Genetic control of premating-isolating behavior: Kaneshiro's hypothesis and asymmetrical sexual selection in pocket gophers. J. Hered. *82* (1991) 192–196.
19 Bullini, L., Speciation by hybridization in animals. Boll. Zool. *52* (1985) 121–137.

20 Bullini, L., and Nascetti, G., Speciation by hybridization in phasmids and other insects. Can. J. Zool. *68* (1990) 1747–1760.

21 Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H., and Bickham, J. W., Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas. Proc. natl Acad. Sci. USA *83* (1986) 9576–9580.

22 Carvalho, G. R., and Wolf, H. G., Resting eggs of lake-*Daphnia*. I. Distribution, abundance and hatching of eggs collected from various depths in lake sediments. Freshwat. Biol. *22* (1989) 459–470.

23 Crease, T. J., and Lynch, M., Ribosomal DNA variation in *Daphnia pulex*. Molec. Biol. Evol. *8* (1991) 620–640.

24 Darwin, C., On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life. John Murray, London 1859.

25 DeMarais, B. D., Dowling, T. E., Douglas, M. E., Minckley, W. L., and Marsh, P. C., Origin of *Gila seminuda* (Teleostei, Cyprinidae) through introgressive hybridization: implications for evolution and conservation. Proc. natl Acad. Sci. USA *89* (1992) 2747–2751.

26 DeMelo, R., and Hebert, P. D. N., Allozymic variation and species diversity in North American Bosminidae. Can. J. Fish. aquat. Sci. *51* (1994) 873–880.

27 DeMelo, R., and Hebert, P. D. N., Founder effects and geographic variation in the invading cladoceran *Bosmina* (*Eubosmina*) *coregoni* BAIRD 1857 in North America. Heredity *73* (1994) 490–499.

28 Dowling, T. E., and Brown, W. M., Allozymes, mitochondrial DNA, and levels of phylogenetic resolution among four minnow species (*Notropis*: Cyprinidae). Syst. Zool. *38* (1989) 126–143.

29 Dowling, T. E., and DeMarais, B. D., Evolutionary significance of introgressive hybridization in cyprinid fishes. Nature *362* (1993) 444–446.

30 Dowling, T. E., and Hoeh, W. R., The extent of introgression outside the contact zone between *Notropis cornutus* and *Notropis chrysocephalus* (Teleostei: Cyprinidae). Evolution *45* (1991) 944–956.

31 Dowling, T. E., Smith, G. R., and Brown, W. M., Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chrysocephalus* (family Cyprinidae): comparison of morphology, allozymes, and mitochondrial DNA. Evolution *43* (1989) 620–634.

32 Ebert, D., Yampolsky, L., and Stearns, S. C., Genetics of life history in *Daphnia magna*. 1. Heritabilities at two food levels. Heredity *70* (1993) 335–343.

33 Ender, A., Identifizierung von nuklearen DNA-Markern in *Daphnia*-Hybrid-Komplexen: RAPD-Analyse. Diploma thesis, J. W. Goethe-University, Frankfurt/Main, Germany 1993.

34 Endler, J. A., Geographic Variation, Speciation, and Clines. Princeton University Press, Princeton 1977.

35 Ferris, S. D., Sage, R. D., Huang, C. H., Nielsen, J. T., Ritte, U., and Wilson, A. C., Flow of mitochondrial DNA across a species boundary. Proc. natl Acad. Sci. USA *80* (1983) 2290–2294.

36 Flößner, D., Kiemen- und Blattfüßer, Branchiopoda, Fischläuse, Branchiura. Tierwelt Dtl. 60, VEB Gustav Fischer Verlag, Jena 1972.

37 Flößner, D., Zur Kenntnis einiger *Daphnia*-Hybriden. Limnologica *23* (1993) 71–79.

38 Flößner, D., and Kraus, K., On the taxonomy of the *Daphnia hyalina-galeata* complex (Crustacea: Cladocera). Hydrobiologia *137* (1986) 97–115.

39 Forbes, S. H., and Allendorf, F. W., Associations between mitochondrial and nuclear genotypes in cutthroat trout hybrid swarms. Evolution *45* (1991) 1332–1349.

40 Ford, E. B., Ecological Genetics, 4th edn. Chapman and Hall, London 1975.

41 Frey, D. G., G. O. Sars and the Norwegian Cladocera: a continuing frustration. Hydrobiologia *96* (1982) 267–293.

42 Frey, D. G., The non-cosmopolitanism of chydorid Cladocera: implications for biogeography and evolution, in: Crustacean Biogeography, pp. 237–256. Eds R. H. Gore and K. L. Heck. Academy of Natural Sciences of Philadelphia, Pennsylvania 1986.

43 George, D. G., and Edwards, R. W., Population dynamics and production of *Daphnia hyalina* in a eutrophic reservoir. Freshwat. Biol. *4* (1974) 445–465.

44 Gießler, S., Mikroevolution und Populationsgenetik im *Daphnia galeata/hyalina/cucullata*-Komplex. Ph.D. thesis, Ludwig Maximilians-Universität München, Germany 1987.

45 Grant, P. R., and Grant, B. R., Hybridization of bird species. Science *256* (1992) 193–197.

46 Grant, V., Plant Speciation, 2nd edn. Columbia University Press. New York 1981.

47 Gyllensten, U., and Wilson, A. C., Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. Genet. Res. *49* (1987) 25–29.

48 Hairston N. G. Jr., and Dillon, T. A., Fluctuating selection and response in a population of freshwater copepods. Evolution *44* (1990) 1796–1805.

49 Hall, D. J., An experimental approach to the dynamics of a natural population of *Daphnia galeata mendotae*. Ecology *45* (1964) 94–112.

50 Hann, B. J., Naturally occurring interspecific hybridization in *Simocephalus* (Cladocera, Daphniidae): its potential significance. Hydrobiologia *145* (1987) 219–224.

51 Harrison, R. G., Hybrid zones: windows on evolutionary process. Oxf. Surv. Evol. Biol. *7* (1990) 69–128.

52 Harrison, R. G. (Ed.), Hybrid Zones and the Evolutionary Process. Oxford University Press, New York 1993.

53 Harrison, R. G., and Rand, D. M., Mosaic hybrid zones and the nature of species boundaries, in: Speciation and its Consequences, pp. 111–133. Eds D. Otte and J. A. Endler. Sinauer Associates, Sunderland, Massachusetts 1989.

54 Harrison, R. G., Rand, D. M., and Wheeler, W. C., Mitochondrial DNA variation in field crickets across a narrow hybrid zone. Molec. Biol. Evol. *4* (1987) 144–158.

55 Hebert, P. D. N., Interspecific hybridization between cyclic parthenogens. Evolution *39* (1985) 216–220.

56 Hebert, P. D. N., Beaton, M. J., Schwartz, S. S., and Stanton, D. J., Polyphyletic origins of asexuality in *Daphnia pulex*. 1. Breeding-system variation and levels of clonal diversity. Evolution *43* (1989) 1004–1015.

57 Hebert, P. D. N., and Crease, T. J., Clonal coexistence in *Daphnia pulex* (Leydig): another planktonic paradox. Science *207* (1980) 1363–1365.

58 Hebert, P. D. N., Schwartz, S. S., and Hrbáček, J., Patterns of genotypic diversity in Czechoslovakian *Daphnia*. Heredity *62* (1989) 207–216.

59 Hebert, P. D. N., Schwartz, S. S., Ward, R. D., and Finston, T. L., Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group. 1. Breeding systems of Canadian populations. Heredity *70* (1993) 148–161.

60 Heiser, C. B. Jr., Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. Evolution *1* (1947) 249–262.

61 Heiser, C. B. Jr., Introgression re-examined. Bot. Rev. *39* (1973) 347–366.

62 Herke, S. W., Kornfield, I., Morgan, P., and Moring, J. R., Molecular confirmation of hybridization between northern pike (*Esox lucius*) and chain pickerel (*E. niger*). Copeia *1990* (1990) 846–850.

63 Hewitt, G. M., Hybrid zones – natural laboratories for evolutionary studies. Trends Ecol. Evol. *3* (1988) 158–167.

64 Hewitt, G. M., The subdivision of species by hybrid zones, in: Speciation and its Consequences, pp. 85–110. Eds D. Otte and J. A. Endler. Sinauer Associates, Sunderland, Massachusetts 1989.

65 Hewitt, G. M., and Barton, N. H., The structure and maintenance of hybrid zones as exemplified by *Podisma pedestris*, in: Insect Cytogenetics, pp. 159–169. Eds R. L. Blackman, G. M. Hewitt and M. Ashburner. Royal Entomological Society of London Symposia 10, Blackwell, Oxford 1980.

66 Hewitt, G. M., Butlin, R. K., and East, T. M., Testicular dysfunction in hybrids between parapatric species of the grasshopper *Chorthippus parallelus*. Biol. J. Linn. Soc. *31* (1987) 25–34.

67 Hobaek, A., and Larsson, P., Sex determination in *Daphnia magna*. Ecology *71* (1990) 2255–2268.

68 Hofmann, W., The late-glacial/holocene *Bosmina* (*Eubosmina*) fauna of lake Constance (Untersee) (F.R.G.): traces of introgressive hybridization. Hydrobiologia *225* (1991) 81–85.

69 Hovanitz, W., Hybridization and seasonal segregation in two races of a butterfly occurring together in two localities. Biol. Bull. *85* (1943) 44–51.

70 Howard, D. J., Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis, in: Hybrid Zones and the Evolutionary Process, pp. 46–69. Ed. R. G. Harrison. Oxford University Press, New York 1993.

71 Howard, D. J., Waring, G. L., Tibbets, C. A., and Gregory, P. G., Survival of hybrids in a mosaic hybrid zone. Evolution *47* (1993) 789–800.

72 Hrbáček, J., Systematics and biogeography of *Daphnia* species in the northern temperate regions, in: *Daphnia*, pp. 37–76. Eds R. H. Peters and R. de Bernardi. Memorie dell'Instituto di Idrobiologia, vol. 45, Verbania Pallanza, Pallanza 1987.

73 Key, K. H. L., The concept of stasipatric speciation. Syst. Zool. *17* (1968) 14–22.

74 Konkle, B. R., and Philipp, D. P., Asymmetric hybridization between two species of sunfishes (*Lepomis*: Centrarchidae). Molec. Ecol. *1* (1992) 215–222.

75 Kraus, F., and Miyamoto, M. M., Mitochondrial genotype of a unisexual salamander of hybrid origin is unrelated to either of its nuclear haplotypes. Proc. natl Acad. Sci. USA *87* (1990) 2235–2238.

76 Lamb, T., and Avise, J. C., Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. Proc. natl Acad. Sci. USA *83* (1986) 2526–2530.

77 Lammens, E. H. R. R., de Nie, J. W., Vijverberg, J., and van Densen, W. L. T., Resource partitioning and niche shifts of bream (*Abramis brama*) and eel (*Anguilla anguilla*) mediated by predation of smelt (*Osmerus eperlanus*) on *Daphnia hyalina*. Can. J. Fish. aquat. Sci. *42* (1985) 1342–1351.

78 Larsson, P., Intraspecific variability in response to stimuli for male and ephippia formation in *Daphnia pulex*. Hydrobiologia *225* (1991) 281–290.

79 Lehman, N., Eisenhawer, A., Hansen, K., Mech, L. D., Peterson, R. O., Gogan, P. J. P., and Wayne, R. K., Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. Evolution *45* (1991) 104–119.

80 Levin, D. A., Hybridization, an evolutionary perspective. Benchmark Papers in Genetics, vol. 11. Dowden, Hutchinson and Ross, Stroudsburg, Pennsylvania 1979.

81 Levin, D. A., and Bulinska-Radomska, Z., Effects of hybridization and inbreeding on fitness in *Phlox*. Am. J. Bot. 75 (1988) 1632–1639.

82 Lewontin, R. C., and Birch, L. C., Hybridization as a source of variation for adaptation to new environments. Evolution *20* (1966) 315–336.

83 Lieder, U., Die Arten der Untergattung *Eubosmina* SELIGO, 1900 (Crustacea: Cladocera, Bosminidae). Mitt. zool. Mus. Berl. *59* (1983) 195–292.

84 Lieder, U., Introgression as a factor in the evolution of polytypical plankton Cladocera. Int. Revue ges. Hydrobiol. Hydrogr. *68* (1983) 269–284.

85 Lieder, U., The possible origin of *Daphnia cucullata procurva* POPPE 1887 in the lakes of the Pomeranian Lakeland by hybridization in the past. Hydrobiologia *149* (1987) 201–211.

86 Lieder, U., The *Bosmina kessleri*-like morphotype of *Eubosmina* in Lake Muskoka, Ontario, Canada, as putative interspecific hybrids. Hydrobiologia *225* (1991) 71–80.

87 Lynch, M., Ecological genetics of *Daphnia pulex*. Evolution *37* (1983) 358–374.

88 Lynch, M., Speciation in the Cladocera. Verh. int. Verein. theor. angew. Limnol. *22* (1985) 3116–3123.

89 Mantovani, B., and Scali, V., Hybridogenesis and androgenesis in the stick-insect *Bacillus rossius-grandii benazzii* (Insecta, Phasmatodea). Evolution *46* (1992) 783–796.

90 Marchant, A. D., Arnold, M. L., and Wilkinson, P., Gene flow across a chromosomal tension zone. I. Relicts of ancient hybridization. Heredity *61* (1988) 321–328.

91 Mayr, E., Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, Massachusetts 1963.

92 McDade, L. A., Hybrids and phylogenetic systematics. 2. The impact of hybrids on cladistic analysis. Evolution *46* (1992) 1329–1346.

93 McGowan, C., and Davidson, W. S., Unidirectional natural hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in Newfoundland. Can. J. Fish. aquat. Sci. *49* (1992) 1953–1958.

94 Moore, W. S., An evaluation of narrow hybrid zones in vertebrates. Q. Rev. Biol. *52* (1977) 263–277.

95 Moore, W. S., and Koenig, W. D., Comparative reproductive success of yellow-shafted, red-shafted and hybrid flickers across a hybrid zone. The Auk *103* (1986) 42–51.

96 Müller, J., Räumliche und zeitliche Variabilität der genetischen Struktur natürlicher Cladocerenpopulationen (Crustacea, Cladocera). Ph.D. thesis, J. Gutenberg Universität, Mainz, Germany 1993.

97 Müller, J., and Seitz, A., Habitat partitioning and differential vertical migration of some *Daphnia* genotypes in a lake. Arch. Hydrobiol. Beitr. Ergebn. Limnol. *39* (1993) 167–174.

98 Müller, J., and Seitz, A., Influence of differential natality and mortality on temporal fluctuations of *Daphnia* genotypes in natural populations, in: Genetics and Evolution of Aquatic Organisms, pp. 342–350. Ed. A. R. Beaumont. Chapman and Hall, London 1994.

99 Patton, J. L., and Smith, M. F., Molecular evidence for mating asymmetry and female choice in a pocket gopher (*Thomomys*) hybrid zone. Molec. Ecol. *2* (1993) 3–8.

100 Powell, J. R., Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila*. Proc. natl Acad. Sci. USA *80* (1983) 492–495.

101 Rand, D. M., and Harrison, R. G., Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. Evolution *43* (1989) 432–449.

102 Rieseberg, L. H., Choi, H. C., and Ham, D., Differential cytoplasmic versus nuclear introgression in *Helianthus*. J. Hered. *82* (1991) 489–493.

103 Rieseberg, L. H., and Wendel, J. F., Introgression and its consequences in plants, in: Hybrid Zones and the Evolutionary Process, pp. 70–109. Ed R. G. Harrison. Oxford University Press, New York 1993.

104 Schierwater, B., Ender, A., Schwenk, K., Spaak P., and Streit, B., The evolutionary ecology of *Daphnia*, in: Molecular Ecology and Evolution: Approaches and Applications, pp. 495–508. Eds B. Schierwater, B. Streit, G. P. Wagner and R. DeSalle. Birkhäuser Verlag, Basel 1994.

105 Schwenk, K., Interspecific hybridization in *Daphnia*: distinction and origin of hybrid matrilines. Molec. Biol. Evol. *10* (1993) 1289–1302.

106 Scribner, K. T., Hybrid zone dynamics are influenced by genotype-specific variation in life-history traits: experimental evidence from hybridizing *Gambusia* species. Evolution *47* (1993) 632–646.

107 Scribner, K. T., and Avise, J. C., Cytonuclear genetic architecture in mosquitofish populations and the possible roles of introgressive hybridization. Molec. Ecol. *2* (1993) 139–149.

108 Shan, R. K., and Frey, D. G., *Pleuroxus denticulatus* and *P. procurvus* (Crustacea, Chydoridae) in North America: distribution, experimental hybridization and the possibility of natural hybridization. Can. J. Zool. *61* (1983) 1605–1617.

109 Solignac, M., and Monnerot, M., Race formation, speciation, and introgression within *Drosophila simulans*, *D. mauritiana*, and *D. sechellia* inferred from mitochondrial DNA analysis. Evolution *40* (1986) 531–539.

110 Spaak, P., Genetical Ecology of a Coexisting *Daphnia* Hybrid Species Complex. Ph.D. thesis, University of Utrecht, The Netherlands 1994.

111 Spaak, P., and Hoekstra, J. R., Clonal structure of the *Daphnia* population in Lake Maarsseveen: its implications for diel vertical migration. Arch. Hydrobiol. Beitr. Ergebn. Limnol. *39* (1993) 157-165.

112 Spaak, P., and Hoekstra, J. R., Life history variation and the coexistence of a *Daphnia* hybrid and its parental species. Ecology *76* (1995) 553-564.

113 Sperling, F. A. H., Evolution of the *Papilio machaon* species group in western Canada (Lepidoptera, Papilionidae). Can. Quaest. Ent. *23* (1987) 198-315.

114 Sperling, F. A. H., and Spence, J. R., Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution *45* (1991) 1370-1383.

115 Spitze, K., Population structure in *Daphnia obtusa* – quantitative genetic and allozymic variation. Genetics *135* (1993) 367-374.

116 Stace, C. A., Hybridization and the plant species, in: Differentiation Patterns in Higher Plants, pp. 115-127. Ed. K. M. Urbanska. Academic Press, New York 1987.

117 Stebbins, G. L., Variation and Evolution in Plants. Columbia University Press, New York 1950.

118 Stebbins, G. L., and Daly, K., Changes in the variation pattern of a hybrid population of *Helianthus* over an eight-year period. Evolution *15* (1961) 60-71.

119 Streit, B., Städler, T., Schwenk, K., Ender, A., Kuhn, K., and Schierwater, B., Natural hybridization in freshwater animals – ecological implications and molecular approaches. Naturwissenschaften *81* (1994) 65-73.

120 Szymura, J. M., and Barton, N. H., Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution *40* (1986) 1141-1159.

121 Taylor, D. J., and Hebert, P. D. N., *Daphnia galeata mendotae* as a cryptic species complex with interspecific hybrids. Limnol. Oceanogr. *37* (1992) 658-665.

122 Taylor, D. J., and Hebert, P. D. N., Habitat-dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species. Proc. natl Acad. Sci. USA *90* (1993) 7079-7083.

123 Taylor, D. J., and Hebert, P. D. N., A reappraisal of phenotypic variation in *Daphnia galeata mendotae* – the role of interspecific hybridization. Can. J. Fish. aquat. Sci. *50* (1993) 2137-2146.

124 Taylor, D. J., and Hebert, P. D. N., Cryptic intercontinental hybridization in *Daphnia* (Crustacea) – the ghost of introductions past. Proc. R. Soc. Lond. B *254* (1993) 163-168.

125 Tegelström, H., Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). J. molec. Evol. *24* (1987) 218-227.

126 Tessier, A. J., Young, A., and Leibold, M., Population dynamics and body-size selection in *Daphnia*. Limnol. Oceanogr. *37* (1992) 1-13.

127 Vijverberg, J., Boersma, M., Van Densen, W. L. T., Hoogenboezem, W., Lammens, E. H. R. R., and Mooij, W. M., Seasonal variation in the interactions between piscivorous fish, planktivorous fish and zooplankton in a shallow eutrophic lake. Hydrobiologia *207* (1990) 279-286.

128 Vijverberg, J., and Richter, A. F., Population dynamics and production of *Daphnia hyalina* LEYDIG and *Daphnia cucullata* SARS in Tjeukemeer. Hydrobiologia *95* (1982) 235-259.

129 Wayne, R. K., and Jenks, S. M., Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. Nature *351* (1991) 565-568.

130 Weider, L. J., Niche breadth and life history variation in a hybrid *Daphnia* complex. Ecology *74* (1993) 935-943.

131 Weider, L. J., and Stich, H. B., Spatial and temporal heterogeneity of *Daphnia* in Lake Constance – intraspecific and interspecific comparisons. Limnol. Oceanogr. *37* (1992) 1327-1334.

132 Weider, L. J., and Wolf, H. G., Life-history variation in a hybrid species complex of *Daphnia*. Oecologia *87* (1991) 506-513.

133 White, M. J. D., Modes of Speciation. W. H. Freeman, San Francisco 1978.

134 Whitham, T. G., Plant hybrid zones as sinks for pests. Science *244* (1989) 1490-1493.

135 Whitham, T. G., Morrow, P. A., and Potts, B. M., Conservation of hybrid plants. Science *254* (1991) 779-780.

136 Wolf, H. G., Population genetic investigations on *Daphnia cucullata* in the Schöhsee at Plön. Verh. int. Verein. theor. angew. Limnol. *22* (1985) 3058-3061.

137 Wolf, H. G., Interspecific hybridization between *Daphnia hyalina*, *D. galeata*, and *D. cucullata* and seasonal abundances of these species and their hybrids. Hydrobiologia *145* (1987) 213-217.

138 Wolf, H. G., Differences in the genetic structure of pond-dwelling and lake-dwelling *Daphnia*. Verh. int. Verein. theor. angew. Limnol. *23* (1988) 2056-2059.

139 Wolf, H. G., and Carvalho, G. R., Resting eggs of lake-*Daphnia* II. In situ observations on the hatching of eggs and their contribution to population and community structure. Freshwat. Biol. *22* (1989) 471-478.

140 Wolf, H. G., and Mort, M. A., Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. Oecologia *68* (1986) 507-511.

141 Wolf, H. G., and Weider, L. J., Do life-history parameters of *Daphnia* as determined in the laboratory correctly predict species successions in the field? Verh. int. Verein. theor. angew. Limnol. *24* (1991) 2799-2801.

142 Zaret, T. M., Strategies for existence of zooplankton prey in homogeneous environments. Verh. int. Verein. theor. angew. Limnol. *19* (1975) 1484-1489.