Doctoral Thesis

Ecological divergence and reproductive isolation between the freshwater snails Lymnaea peregra (Müller 1774) and L. ovata (Draparnaud 1805)

Author(s):
Wullschleger, Esther

Publication Date:
2000

Permanent Link:
https://doi.org/10.3929/ethz-a-004099299

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Ecological divergence and reproductive isolation between the freshwater snails *Lymnaea peregra* (Müller 1774) and *L. ovata* (Draparnaud 1805)

A dissertation submitted to the
Swiss Federal Institute of Technology Zürich

for the degree of
Doctor of Natural Sciences

Presented by

Esther Wullschleger
Dipl. Biol. Univ. Zürich
born October 22nd, 1966
in Zürich, Switzerland

Submitted for the approval of
Prof. Dr. Paul Schmid-Hempel, examiner
Dr. Jukka Jokela, co-examiner
Prof. Dr. Paul Ward, co-examiner

2000
“J’ai également recueilli… toute une série de formes de *L. ovata* dont les extrêmes, très rares cependant, sont bien voisins de l’espèce de Müller. Mais je dois dire que, si dans une colonie de *L. ovata*, on constate parfois de ces individus extrêmes qui ne sont guère discernables de *L. peregra*, je n’ai jamais observé une seule colonie de cette dernière espèce qui présente le fait inverse, c’est-à-dire des individus qui pourraient être confondus avec l’espèce de Draparnaud. Il semble donc bien qu’il y ait deux types spécifiques, l’un polymorphe, *L. ovata*, don’t certains exemplaires simulent par convergence le second, *L. peregra*, celui-ci ne montrant pas ou bien peu de variabilité dans la direction du premier.”

J. Favre (1927)
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Summary

Ecological divergence may proceed to speciation if reproductive isolation ultimately evolves between populations which exploit different resource environments. This scenario, where reproductive isolation evolves through natural selection against the production of hybrids, contrasts with the more widely occurring scenario of allopatric speciation. The ecological speciation hypothesis, and in particular the hypothesis of sympatric species formation, have received increasing interest in current evolutionary biology. Most importantly, clear empirical examples of reinforcement of premating isolation in sympatry remain scarce. In all, the understanding of ecological speciation may benefit from additional case studies covering a wide variety of taxa.

In this thesis, I aimed to determine the stage and the possible agents of divergence in a pair of closely related freshwater snails which show several attributes that may be indicative of ecological speciation. *Lymnaea peregra* and *L. ovata* occur in a largely overlapping range across Europe. Within this range, their distribution appears geographically irregular and varies in correlation with habitat characteristics. In the first part of my study, I investigated whether the snail types differ in habitat-specific distribution across eastern Switzerland. As predicted by previous observations, *Lymnaea peregra* was more common in smaller, more temporary habitats and at higher altitude. As a second aspect, this field survey showed that trematodes (a group of highly pathogenic parasites that are of major evolutionary importance to their molluscan intermediate hosts) do not play a significant role in driving the spatial separation of the two snail types. However, as shown in the second part, *Lymnaea peregra* and *L. ovata* exhibit genetically based divergence in significant life-history traits. Specifically, *L. peregra* grew faster as juveniles and had a more iteroparous reproductive schedule. These traits may be beneficial in small and temporary freshwater habitats which are prone to unpredictable desiccation. On the other hand, shell form appeared to be considerably plastic. Both snail types turned into a narrow, *peregra*-like shell form in only few laboratory generations. As shown in the third part, the two snail types show evidence of reproductive character displacement in a sympatric field site. In allopatric populations, assortative mate choice (favoring intraspecific matings) was expressed by some, but not all populations. Reproductive character displacement may be explained by the cost of mate choice errors and maladaptive hybridization. In conclusion, the present evidence indicates that the snail types are sufficiently isolated to justify species status. Because the stage of divergence is advanced, the present results cannot be taken as conclusive evidence that ecological causes have been initiating divergence, although distributional data suggest that this remains the more likely explanation.
Zusammenfassung


General Introduction and Thesis Outline

Ecological divergence, ecological speciation and adaptive radiation

Ecological divergence is the splitting of a lineage as a consequence of divergent selection between populations which exploit different resources or environments. In the extreme case, ecological divergence may lead to ecological speciation, in that reproductive isolation ultimately evolves from contrasting selection pressures between populations exploiting different resource environments (Schlüter 1996b). This mechanism contrasts with the apparently more widely occurring scenario of allopatric speciation. In the second case, speciation occurs when geographical barriers inhibit interbreeding between two populations, resulting in reproductive isolation as a pleiotropic by-product of increasing genetic divergence between the two allopatric populations (Mayr 1942; Dobzhansky 1951; Rice and Hostert 1993).

The core principle of the ecological speciation hypothesis is that pre- and postzygotic isolation builds up between populations that are climbing separate adaptive peaks which correspond to distinct ecological niches, such that hybrid offspring which are intermediate in phenotype would fall between the peaks and therefore be removed by selection (Hatfield and Schluter 1999). A number of studies substantiate that ecological divergence has initiated speciation in postglacial fishes of the northern temperate zone (Schlüter 1996b; Gislason et al. 1999; Skulason et al. 1999; Turgeon et al. 1999). In these cases, the development of feeding polymorphisms, that is, alternative feeding strategies, in newly available habitats at recolonization after glaciation seems to have been the causal agent of divergence (see Schluter 1995; Schluter 1996a). Further studies strengthen the view that the availability of niche space may be a crucial element favoring ecological speciation (as an example, see Galis and van Alphen 2000). The observation that allopatric divergence also appears more likely when populations adapt to different environments rather than under the influence of genetic drift alone (cf. Funk 1998), lends further support to the hypothesis that the availability of niche space favors divergence.

To my knowledge, corresponding studies on ecological divergence of freshwater invertebrates of the northern temperate zone are thus far absent. Since freshwater invertebrates may disperse more easily than fish and colonize new freshwater habitats earlier, it would be of interest to know whether adaptive radiation has been as common in this group as in postglacial fishes. From subfossil shell findings and the relative distribution of alternative shell forms across time and space, it has been presumed that some species groups of
freshwater snails (the *Valvata piscinalis-macrostoma-pulchella* group and the *Lymnaea (Radix) peregra-ovata-ampla* group; personal communication N. Thew) may have initiated ecological divergence during or following glaciations. In the case of freshwater pulmonate snails, the evolution of feeding polymorphisms appears unlikely, since they are relatively unspecific Aufwuchs, plant and detritus grazers (Knecht and Walter 1977; Dillon 2000). Other trait groups which are more variable in these snails, and may therefore be subject to divergence, are shell morphology and life-history strategies. These traits could vary, for example, dependent on environmental factors such as habitat permanence, desiccation risk, availability of food, water movement, or predation.

Reinforcement of premating isolation

A further major area of contemporary research around the scenario of ecological speciation is the question of whether assortative mating could be reinforced between two diverging populations in sympatry as a response to selection against hybridization (reinforcement of mate choice hypothesis, e.g. Butlin 1989; Howard 1993; Hostert 1997; Kirkpatrick and Servedio 1999; Noor 1999; Kulathinal and Singh 2000). Because positive assortative mating is an inevitable by-product of divergence in habitat choice (Barton et al. 1988), strong habitat preferences may already lead to a certain degree of reproductive isolation. This has been shown, for example, in systems where host-specific herbivorous insects exhibit host-assortative mating, in that matings occur on the preferred host plant (Bush 1969; Feder et al. 1990; Craig et al. 1993; Via 1999). In systems where alternative habitat preferences are sufficiently strong in restricting gene flow to allow population divergence, an old dilemma of the sympatric speciation model is circumvented: A major objection to this model has been that there must be evolution of genes both for host or habitat preference and for performance in the new host or habitat, two different traits whose linkage may be broken up by gene flow and recombination (Felsenstein 1981).

The reinforcement of mate choice hypothesis has received recent theoretical support (e.g. Liou and Price 1994; Dieckmann and Doebeli 1999), but empirical evidence suggests that its occurrence might be restricted to certain ecological or demographic situations (e.g. Coyne and Orr 1989; Johannesson et al. 1995; Coyne and Orr 1997; Hellberg 1998; Schluter 1998). To reveal its relative importance in speciation under natural conditions, more studies on systems in a stage of early divergence have recently been called for (Bridle and Jiggins 2000). However, the study of reinforcement of premating barriers is
complicated by the fact that reproductive character displacement in sympathy may also occur between two species which had previously speciated in isolation and met in secondary contact. Yet studies of reproductive character displacement between taxa in an advanced stage of divergence may also shed light on the actual process of behavioral isolation in particular systems. Rather than being a unique trait, behavioral isolation may arise through a mismatch between the complex courtship signals that are used by each of two diverged species, as has been shown in *Drosophila* (Boake 2000). Reproductive character displacement between distinct species has mostly been documented for animal groups with pronounced mate signalling traits (e.g. Marquez and Bosch 1997; Pfennig 2000).

The present system

Overall, the understanding of ecological speciation may benefit from additional case studies on systems in varying stages of segregation, which may help to clarify the restrictiveness of conditions needed for its occurrence. Here, I study some selected aspects of ecological divergence and race formation in two closely related freshwater snails which appear to be in a stage of speciation, by investigating some of the most important attributes indicative of ecological speciation: the possibility of habitat specialization, the presence of disruptive selection, and the possibility of rapid evolution of assortative mating in a sympatric location. In particular, I studied whether the two snail types show evidence of divergent habitat preferences, whether they meet different communities of trematode parasites and unequal infection risks in their respectively preferred habitat types, whether morphological and life-history variation is genetically based and larger between than within the snail types, and, finally, whether there is evidence for assortative mating in sympatric and in allopatric sites. My results showed that the snails indeed have distinct habitat preferences, but these seemed independent of prevalences of trematode infection. Furthermore, there was evidence for genetically based differences in significant life-history traits (juvenile growth and reproductive scheduling). Shell morphology, although its variation is disruptive in natural populations and intermediate forms are exceedingly rare, seems considerably plastic: Both snail types developed a similar shell form in only few laboratory generations. Shell form and life-history traits correlated with the snails' respective habitat preferences as predicted by selection regimes, suggesting that the differences may be functionally adaptive. Finally, evidence of assortative mating and reproductive character displacement in sympathy strengthens the view that the snail types represent distinct taxons which are reproductively isolated at least to some extent.
Lymnaea (Radix) peregra/ovata as a study system

Lymnaeids belong to a group of freshwater pulmonate snails (Pulmonata: Basommatophora) which commonly inhabit a variety of freshwater habitats and have a cosmopolitan distribution. Typically, Lymnaeid species show a high degree of phenotypic plasticity (in particular, high intraspecific variation in shell morphology), but low morphological differentiation between closely related species. High levels of adaptive plasticity have been suggested to enable these snails to colonize rapidly novel habitats (Russell-Hunter 1978). Because of this morphological plasticity, the evolutionary history of Lymnaeid species is relatively difficult to reconstruct from fossil findings.

Relationship to Lymnaea auricularia

It is highly probable that the snails considered here, Lymnaea (Radix) peregra/ovata, evolved from forms similar to Lymnaea (Radix) auricularia (see Hubendick 1951). Lymnaea auricularia has a greater eastern distribution and apparently originates from the Asian region of the continent, but does almost completely cover the range of L. peregra/ovata; the latter shows its greatest degree of variation in western Europe, which may indicate its European origin (Hubendick 1951). Most probably, the separation of L. peregra/ovata and L. auricularia occurred during one of the glacial periods, with L. peregra/ovata being confined to the west side of an ice barrier, and L. auricularia to the east (Wright 1959). This view is supported on one hand by the relatively greater abundance of L. peregra/ovata in western Europe, and on the other hand by the finding of fossil L. peregra/ovata in Britain in interglacial deposits, while L. auricularia has been found only in post-glacial British deposits (Zeuner 1945). Continental European pleistocene deposits also carried L. peregra/ovata, while L. auricularia was not reported (Lozek 1976; Schlickum and Strauch 1979; Cech et al. 1997; Wendebourg 1997). However, L. auricularia and L. ovata closely resemble each other in shell form, and misidentifications may be possible. Lymnaea peregra/ovata and L. auricularia show clear differences in internal anatomy (Hubendick 1945; Hubendick 1951), and although the two species occasionally copulate in natural situations, anatomically intermediate forms (suggesting the occurrence of hybridization) appear to be absent at least in Zürichsee, Switzerland, where the two species are sympatric (Burla and Speich 1971). To my knowledge, species status between Lymnaea peregra/ovata and L. auricularia has never been questioned.
The shell forms *peregra* and *ovata*

*Lymnaea peregra/ovata* has two distinct shell forms, called *ovata* and *peregra*, which are sometimes considered as distinct species (e.g. Fechter and Falkner 1990; Glöer and Meier-Brook 1998; Turner et al. 1998), but also commonly seen as morphs of one species either named *L. peregra* or *L. ovata* (e.g. Mouthon, personal communication; Hubendick 1945; Økland 1990). The two shell forms have been said to show no differentiation in internal anatomy, which has been taken as a major criterium for subspecies status (Hubendick 1945), although some authors suggested that they can be distinguished by parts of the soft body (Favre 1927; Glöer and Meier-Brook 1998). Because of the taxonomic confusion surrounding the two shell forms, it has hardly been possible to find sufficient and reliable information on their fossil history in relation to each other, as for example on the sequence of their occurrence in the fossil record. However, both shell types have been reported from pleistocene sediments (e.g. Thiemenmann 1950).

Today, *L. peregra* and *L. ovata* overlap in geographical distribution across the main part of their range (cf. Turner et al. 1998). Within this range, however, the distribution of the two forms is geographically irregular (Hubendick 1951; Kerney 1999). Distribution may rather be habitat-dependent (cf. Økland 1990), and the snail types differ in microhabitat preferences (Wullschleger and Ward 1998). Sympatric occurrence in the same habitat is relatively rare (pers. observation Wullschleger). Although the phylogeography is not well resolved for this system, the current distribution of the two snail types may suggest that ecological mechanisms have been responsible for their divergent evolution, allowing for a sympatric origin. Kondrashov and Mina (1986) suggested that initial sympatry is likely in animals which colonize a new habitat, while at a later stage of colonization two types have segregated enough such that they may show a patchy distribution of several allopatric populations (Kondrashov and Mina 1986). Furthermore, the low differentiation of the two snail forms in several characters (enzyme variation, shell morphology) may indicate that they should be considered as ‘early’ species, whose speciation, if at all, has been completed relatively recently.

In the following, I present the separate questions I aimed to address within the frame of this work. Finally, I will conclude with a general discussion of the obtained results.
Thesis outline

1. The role of habitat preferences

"With habitat and food selection – behavioral phenomena – playing a major role in the shift into new adaptive zones, the importance of behavior in initiating new evolutionary events is self-evident."
Mayr 1963

A first step in ecological divergence and ecological speciation may be the genetic fixation of differences in habitat preferences. Habitat preference is especially significant in this context because it determines the regime of natural selection on loci that affect adaptation to a particular environment (Jaenike and Holt 1991). The linkage of genetic differences with habitat choices has been documented for a variety of genetic polymorphisms (cf. Jones 1980; Jaenike and Holt 1991), and when habitat preference is coupled with mate choice, progress toward nonallopatric speciation may occur under a broad range of biological conditions (Diehl and Bush 1989). Habitat choice may most likely initiate the process of speciation in systems with strong habitat specialization (such as phytophagous insects, or host-specific parasites), which comprise a major part of documented empirical examples of ecological divergence or ecological speciation (e.g. Bush 1969; Feder et al. 1990; Craig et al. 1993; Via 1999). Microhabitat preferences have also been suggested to be involved in the formation of incipient reproductive isolation in two shore snail morphs which may be in a stage of incipient speciation (Johannesson et al. 1995). In this context, it is important to note that other factors may as well, or additionally, lower encounter rates with possible mates in sympathy, such as differences in activity patterns (e.g. Dobzhansky 1973; Miyatake and Shimizu 1999).

The present work was motivated by the results of my masters thesis, where I had investigated whether L. peregra and L. ovata differ in habitat preferences, and whether habitat choice could be explained by a selective advantage of shell form under the respective habitat conditions (Wullschleger and Ward 1998). In particular, I aimed to test the prediction that the narrow shell form of L. peregra is favored in locations with high desiccation risk, such as in the shallowest regions of a flat shore. Generally, a high risk of desiccation appears to favor narrower shell forms which allow restriction of water loss, as suggested by studies on high intertidal molluscs (Vermeij 1971b; Vermeij 1973; Etter
1988b; Chapman 1995). The results of a field survey indicated that *L. peregra* was more common in the shallower regions of a flat, vegetated shore at Seéalpsee (Switzerland) which is inhabited by both snail types (MANOVA on depth class by population, df=1, MQ=9.19, F=25.94, P=0.007, cf. Wullschleger 1994).

To exclude alternative explanations for such distributional patterns (for example, an effect of competition or colonization history), I also attempted to demonstrate habitat preference in a laboratory choice setting during my masters thesis. In this, samples of each shell form were allowed to independently choose for substrate type (hard substrates versus mud and vegetation) and water depth. Additionally, I tested snails from a neighboring population of *L. ovata*, which inhabited an adjacent stony shore with a steeper gradient. The results showed that all snails were about equally likely to be found on hard substrates, but *L. peregra* was significantly more likely than both *L. ovata* populations to be found on mud or vegetation (Wullschleger and Ward 1998). Furthermore, *L. peregra* was commoner in the three shallowest depth classes, while *L. ovata* from the vegetated shore preferred somewhat intermediate depths, and *L. ovata* from the stony shore markedly preferred the deepest depth (Wullschleger and Ward 1998). Since *L. peregra* and *L. ovata* from the vegetated shore were collected from the same area, their differential habitat choices in the laboratory may indicate that habitat preference could be partially under genetic control. However, the variation between *L. ovata* from the vegetated shore and *L. ovata* from the stony shore indicates that there is also pronounced environmentally induced variation. Unfortunately, laboratory-based breeding of the populations used in this experiment did not yield sufficient individuals to repeat the experiment with laboratory-raised snails.

In total, these results led to the hypothesis that *Lymnaea peregra* and *L. ovata* may show divergence in habitat-specific distribution. In the first part of my work, I investigated whether populations from a large part of Switzerland differ in habitat-specific distribution.
2. The potential role of parasites

It has often been proposed that species interactions are a major source of adaptive radiation (e.g. Darwin 1859; Thompson 1994; Pellmyr and Leebens-Mack 2000). It may therefore be of importance in the study of ecological divergence to consider associations of diverging lineages with coevolving organisms. Because of their comparatively close association, host-parasite systems may be of particular interest in this respect. The most virulent parasites of freshwater snails are the larval stages of trematodes, which mostly use snails as obligate intermediate hosts. These parasites could impose a strong selection pressure on snail populations, because trematode infection usually leads to castration of the molluscan intermediate host (Kuris 1974; Minchella 1985; Minchella et al. 1985). That trematodes can have a significant impact on the population structure of snail intermediate hosts is particularly obvious if infection risk varies spatially or temporally (e.g. Brown et al. 1988; Jokela and Lively 1995). Due to their life stages external of molluscan intermediate hosts, the presence and abundance of trematodes in specific habitats depends on a variety of factors (reviewed in Esch and Fernandez 1994), some of which may be habitat-specific (e.g. Ginecinskaja 1971; Appleton 1983; Wilson et al. 1996) independently of snail density.

As a second aspect, the first part of my study tests the idea that habitat-specific risk of parasitism may have led to the exclusion of susceptible host types from parasite-rich environments, and to the divergence of the two snail types in spatial distribution. I surveyed field populations of both snail types and recorded a number of habitat characteristics for each site. A sample of collected snails was taken to the laboratory to determine infections and parasite types. A discriminant analysis, with the habitat characteristics combined to two habitat dimensions, revealed that the two snail types differ in habitat-specific distribution. However, the parasitological survey showed that the different parasite types did not differ radically in habitat-specific distribution among each other. I also found no evidence that prevalence of infection was correlated with the relative distribution of the two snail types. Therefore, the idea that the prevalence of parasitism might explain differences in the habitat distribution of the snail types is not supported by the obtained results, and trematode parasites do not seem to be responsible for the habitat-specific divergence between *L. peregra* and *L. ovata.*
3. Divergence in shell form and life-history traits

The hypothesis of ecological speciation relies on divergent selection regimes which may lead to alternative morphological and/or life-history adaptations between two segregating lineages. However, another strategy to populate a variable environment and a broad ecological niche is the maintenance of phenotypic plasticity. If there is segregation for habitat use, habitat-specific traits or adaptations should become genetically fixed, and divergent evolution could proceed to speciation (cf. Mokady et al. 1999). Alternatively, adaptations or traits which do not promote habitat specificity will probably lead to some degree of phenotypic plasticity, in response to factors other than habitat characteristics (Mokady et al. 1999). In this second case, ecological speciation may be unlikely, because the populations involved would not develop genetic differences which would be correlated with habitat choice. Revealing genetically based differences between two lineages that differ in habitat distribution, and, if possible, investigating their adaptive value in relation to habitat factors, is therefore crucial to the study of ecological speciation.

In freshwater Lymnaeids, shell form and life history traits are characterized by considerable intraspecific variation, and at least shell form is highly plastic (e.g. Boycott 1938; Russell-Hunter 1978). Therefore, these two trait groups may most likely be subject to divergence under the influence of contrasting selection pressures. Shell form is influenced by habitat factors such as current and strength of water movement, favoring broader shells (Lam and Calow 1988; Trussell 1997), or desiccation risk, favoring narrow shell apertures (Vermeij 1971b; Vermeij 1973; Etter 1988b; Chapman 1995). Life-history traits are often divergent among populations inhabiting permanent ponds and populations living in temporary water bodies, where the breeding season is shortened and high rates of unpredictable mortality may occur (e.g. Brown et al. 1985), although other factors such as parasitism by trematodes (cf. Chapter 1) may also have a profound influence on life-history evolution.

In the second chapter, I ask whether shell form and life-history strategies show greater between-type than within-type variation in *L. peregra* and *L. ovata*. If there are genetically based differences, this may be explained by differential selection through habitat-dependent factors. For this purpose, I raised offspring from two populations of each snail type in the laboratory. A common garden life-history experiment with these snails revealed genetic divergence in juvenile growth and in reproductive scheduling. However, both *L. peregra* and *L. ovata* showed considerable morphological plasticity in response to laboratory conditions, and both converged to a similar, narrow shell form within only two
laboratory generations. This indicates that shell form responds quickly to environmental change, which may largely be due to phenotypic plasticity.

4. The role of assortative mating

Reproductive isolation between two segregating lineages can evolve as a byproduct of ecological divergence (e.g. Rice and Salt 1990), or as a response to selection which acts directly upon reproduction (e.g. Schluter 1998; Charlesworth and Charlesworth 2000). If the second is true, it may be expected that avoidance of interspecific matings is enhanced in sympatric populations compared to allopatric populations. This pattern, known as reproductive character displacement, has been attributed to the strengthening of mating barriers to avoid maladaptive hybridization and waste of reproductive effort (Noor 1999), a process that is usually termed reinforcement of premating isolation (e.g. Loftus-Hills and Littlejohn 1992). Reproductive character displacement has been documented at the intraspecific level and may be involved in the sympatric formation of species barriers in at least some cases (e.g. Coyne and Orr 1989; Noor 1995; Coyne and Orr 1997; Rundle and Schluter 1998; but see Ritchie et al. 1989; Ritchie et al. 1992; Gregory et al. 1998).

Most studies of reproductive character displacement involve species or subspecies pairs with highly differentiated mating signals, which allow a concomitant analysis of signalling traits and mate choice. For example, taxa with acoustic signalling (Marquez and Bosch 1997; Castellano et al. 1998), with more or less complex courtship behaviour sequences (Boake 2000), or taxa with a strong degree of sexual selection (Price 1998) may be overrepresented in this field of research. In the simultaneously hermaphroditic lymnaeid snails, however, active mate signalling seems to be absent (Dillon 2000). As an aspect of choice, ‘females’ can discriminate against non-suitable sperm donors by displaying rejection behavior, such as shell shaking (e.g. Rudolph 1979; DeWitt 1991; Dillon 2000). Mate choice in pulmonate snails has been reported to depend on the relative sizes of the involved partners (e.g. DeWitt 1996), on parasitic infections (Rupp 1996), and on whether possible partners originate from sympatric or allopatric populations (Rupp and Woolhouse 1999). This last observation suggests that selection may favour the maintenance of local adaptations (Rupp and Woolhouse 1999). Overall, these snails also offer an opportunity to test for assortative mating in organisms which have not evolved differentiated mating signals that would enhance the probability of a population split via assortative mating.
I conducted a series of laboratory experiments to test whether mating is 'species'-assortative or population-assortative, i.e. whether 'snail type' or 'sympatric origin' had a higher effect on mate choice. The first possibility would indicate that differentiation between the snail types is advanced, and incipient or completed speciation may be a possible explanation for this pattern. The second possibility would indicate that populations within the types are differentiated to an extent that choosing locally adapted partners is favored. In the second case, the two snail types may in fact represent a complex of differentiated forms, which have evolved local adaptations but may generally still be capable of interbreeding. In particular, I was interested in testing whether the case of stable sympatry at Sealpsee (Switzerland), where the two snail types showed differential habitat preferences (Wullschleger and Ward 1998), shows evidence of enhanced reproductive isolation, as indicative of reproductive character displacement. As enzyme analysis using a diagnostic enzyme locus revealed no hybrids (unpublished data), extensive hybridization may be unlikely at this site. My results suggest that mating is 'species'-assortative in some, but not all allopatric population combinations, and that reproductive isolation is enhanced in snails from the one sympatric site examined. The second finding indicates reproductive character displacement in sympatry.
Does spatial variation in trematode infection risks influence habitat distribution of two closely related freshwater snails?

(Esther Wullschleger and Jukka Jokela)

Summary

Parasitism may be an important factor determining the geographic distribution of closely related species. A habitat-specific risk of parasitism may lead to an exclusion of susceptible host types from parasite-rich environments, and promote speciation if it leads to reproductive isolation between susceptible and resistant types. We surveyed populations of the freshwater snail *Lymnaea peregra* for differences in habitat preference and trematode parasitism between its two distinct shell morphs, *L. ovata* and *L. peregra*. We surveyed 58 populations (43 *L. ovata*, 15 *L. peregra*). At each location we recorded an array of habitat characteristics that were summarized using a Nonlinear Principal Component Analysis (NPCA). This yielded two orthogonal habitat-score variables. Discriminant analysis with these habitat-score variables indicated that the snail morphs differed in their habitat distribution. *L. ovata* preferred larger, more permanent natural habitats, surrounded by forests, while *L. peregra* was found more often at higher altitude, in non-permanent habitats, often surrounded by meadows. The snails were parasitized by four cercarial types of castrating trematodes. The morphs had a similar prevalence of infection by each of the parasite types, with one exception: Monostomid cercaria were found at a higher prevalence in *L. ovata* than in *L. peregra*. However, monostomes were rare parasites, and the difference was not significant if only populations infected by monostomes were used in the comparison. Our results indicate that variation in the overall prevalence of infection seems to be independent of snail morph, and do not support the idea that a difference in the rate of parasitism might explain differences in the habitat distribution of these snail morphs.

1Published in *Oecologia* 121 (1999): 32-38
Introduction

A habitat-specific risk of parasite infection may exclude some host genotypes from parasite-rich environments. Although the role of parasites in competitive interactions between closely related species is widely recognized (Haldane 1949; Barbehenn 1969; Cornell 1974; Price et al. 1988; Durrer and Schmid-Hempel 1995; Yan and Stevens 1995; but see Hanley et al. 1998; Hudson and Greenman 1998; Yan et al. 1998), their role in speciation events (by enhancing the reproductive isolation between host types) remains largely speculative (e.g., Wheatley 1980). It has been suggested that the allopatric geographic distribution of closely related species may result from the action of shared parasites rather than from resource or interference competition (Price et al. 1986), but empirical data testing this hypothesis are scarce. The idea that susceptible hosts would find refuge in parasite-free environments, and become spatially isolated from resistant hosts, relies on three critical assumptions: (1) that some habitats are not suitable for the parasite, even if the host is present, (2) that resistance carries costs in a parasite-free environment, and (3) that gene flow between the sites is low. If these conditions are met, then parasitism may lead to geographic separation of resistant and susceptible hosts.

Since the host is actually the habitat for the parasite, it is commonly thought that parasites have few habitat requirements apart from the presence of suitable hosts. Therefore, condition (1) above may be rarely met. However, parasites that require transmission between several host species (i.e., that have complex life-cycles) are restricted to habitats where all host species are sympatric. If parasites are excluded from some of the habitats that are suitable for the intermediate host, it is possible to test the idea that susceptible and resistant hosts may be found in different habitats. The second condition above, assuming that resistance implies fitness costs, has been studied extensively. Empirical studies generally support the view that resistance carries costs (Boots and Begon 1993; Mitchell-Olds and Bradley 1996; Oppliger et al. 1996; Kraaijeveld and Godfray 1997; Fellowes et al. 1998; Mauricio 1998).

We chose a snail-trematode system to test whether parasitism plays a role in the geographic separation of host types. More specifically, we ask if two distinct shell morphs of the freshwater snail Lymnaea peregra Mueller (1774) differ in their habitat preference and rate of parasitism. The taxonomic status of these morphs, hereafter called L. ovata and L. peregra, is unresolved; they may be in a process of incipient speciation. The snails are commonly considered as variants of the same species, because they do not differ in internal anatomy (Hubendick 1951), and because differences in shell morphology have
been found to correspond with enzyme variation only in some, but not in all populations (Lam and Calow 1988; Evans 1989). However, consistent variation between the morphs has been found in shell morphology, population genetic structure, parasite susceptibility (Ward et al. 1997), and habitat choice (Wullschleger and Ward 1998). In a laboratory experiment, *L. peregra* preferred mud over hard substrate, but showed no preference for low or high water level, while *L. ovata* did not show a preference for a specific substrate type, but significantly preferred deeper water (Wullschleger and Ward 1998).

If these snail morphs differ in their habitat distribution because of unequal parasite susceptibility, parasitism may enhance their reproductive isolation. The parasites considered here, digenetic trematodes, are highly pathogenic to their snail intermediate hosts, usually castrating the infected hosts (Kuris 1974; Minchella 1985; Minchella et al. 1985). Importantly for our purposes, the presence and abundance of trematodes in specific habitats depends on a variety of factors (Esch and Fernandez 1994), some of which are independent of snail density. For example, the main determinant for the presence of trematodes in a specific habitat is probably the geographic distribution of the definitive hosts (Appleton 1983; Fernandez and Esch 1991). The abundance of definitive hosts may largely determine the risk of infection for the intermediate snail hosts in a specific habitat. In general, trematodes appear to be more common in shallow water (Appleton 1983; Jokela and Lively 1995; Wilson et al. 1996), in water bodies with slow current or still water (Ginetinskaya 1971), and in areas where human activity is low (Keas and Blankepoor 1997; Lafferty 1997). Therefore, the broad habitat range of these snails probably includes habitats which differ in quality for the parasites.

In the present study we measured and characterized habitat preference of the snail morphs using data from natural populations. We then associated habitat characteristics with the risk of trematode infection. We found that the snail morphs differ in habitat preferences, but we found no support for the idea that parasitism would affect the host types differently.
Materials and methods

Study system

*L. (Radix) peregra* (Mueller 1774) has two distinct shell types, referred to as *L. ovata* and *L. peregra*. Both morphs are common across Switzerland and inhabit a large variety of freshwater habitats. The shell of *L. peregra* is narrow and elongate, while *L. ovata* has a more compact shell and a wider opening.

Digenean trematodes have complex life cycles usually with two or three host species. The first intermediate host is a mollusc, in this case *Lymnaea*. The second intermediate host can be a vertebrate or an invertebrate, while definitive hosts are vertebrates. *L. ovata* and *L. peregra* both serve as intermediate hosts for a group of trematodes. Some trematodes are described to species, but several species remain undescribed. However, cercarial types (transmission stage of the worms that is produced in the snail host) can be treated as operational taxonomic units. Following cercarial descriptions of communities inhabiting Switzerland and nearby countries (Luehe 1909; Dubois 1928; Meyer 1964), we distinguished the following cercarial types: monostomid cercaria, xiphidiocercaria, furcocercaria, and echinostomatid cercaria. For each of these units we found a main type which occurred commonly, while some rare types occurred occasionally in monostomes and in furcocercariae.

Field collections

During the summer season 1997, we chose field sites from eastern Switzerland (Fig. 1) by locating freshwater habitats (ditches, creeks, streams, ponds, lakes) from topographic maps. If either of the snail morphs was found when the site was visited, a sample was collected, and the site was included in the study. Selection of sites was constrained to non-protected freshwater habitats with unrestricted access. From a total of 135 visited sites, 43 had a sufficient number of *L. ovata* and 15 had sufficient numbers of *L. peregra*. At least 20, if possible 50-100 snails were collected from each site. The snails were picked by hand, i.e., all snails were collected from the shallow littoral zone. At each site, the snails were collected from a large area of the same microhabitat type, so that any local infection hotspots would not bias the prevalence of infection estimates. Parasite infections
were determined by crushing snails between two glass plates under a dissecting microscope. This method is very reliable, revealing also early stages of infection. The samples were processed on the day they were collected, or at most 1 day later.

We recorded the following habitat characteristics from each site: altitude, substrate, type of surrounding landscape (which may influence the presence of definitive hosts), habitat permanence, current speed, habitat size, and slope of the habitat (indicating whether the snails had access to deep water). These measures were taken at an ordinal or at a categorical scale (see Table 1).
Table 1. Classification of habitat variables recorded at each sample site. *NPCA* indicates how the variable was coded in the nonlinear principal components analysis: *Ordinal* refers to ordinal scale of measurement, *Multiple Nominal* categories of the variable were assigned NPCA scores independently from the scores of the other categories, *Numeric* indicates that the variable was entered as a discrete numeric variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Name of category</th>
<th>Description</th>
<th>NPCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surrounding</td>
<td>Forest</td>
<td>Natural habitats, usually small forests</td>
<td>Multiple nominal</td>
</tr>
<tr>
<td></td>
<td>Farm</td>
<td>Agricultural, usually meadows</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Built</td>
<td>Human built, village, or industrial area</td>
<td></td>
</tr>
<tr>
<td>Current speed</td>
<td>Still</td>
<td>Standing water</td>
<td>Ordinal</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>Slow current</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast</td>
<td>Fast current</td>
<td></td>
</tr>
<tr>
<td>Shore slope&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Shallow</td>
<td>Wide area between 0-50 cm</td>
<td>Ordinal</td>
</tr>
<tr>
<td></td>
<td>Steep</td>
<td>Small area between 0-50 cm</td>
<td></td>
</tr>
<tr>
<td>Altitude</td>
<td></td>
<td>Altitude in meters</td>
<td>Numeric</td>
</tr>
<tr>
<td>Substrate</td>
<td>Mud</td>
<td>Soft sediment</td>
<td>Multiple nominal</td>
</tr>
<tr>
<td></td>
<td>Hard</td>
<td>Large boulders or cement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>Small stones mixed with soft sediment</td>
<td></td>
</tr>
<tr>
<td>Habitat permanence</td>
<td>Not permanent</td>
<td>Small ponds or creeks which may completely dry</td>
<td>Multiple nominal</td>
</tr>
<tr>
<td></td>
<td>Shore</td>
<td>Shallow shores which may dry but are in contact with deeper water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permanent</td>
<td>Permanent water bodies</td>
<td></td>
</tr>
<tr>
<td>Habitat size</td>
<td>Small</td>
<td>Ponds up to 10 m$^2$, creeks up to 2 m</td>
<td>Ordinal</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>Ponds, lakes, creeks, and rivers larger than above</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Snails were collected from shallow littoral (<50cm); this variable indicates whether snails had access to deeper water.
Fig. 1 Map of sampling sites in Switzerland
Data analysis

We used a Nonlinear Principal Components Analysis (NPCA) to generate orthogonal habitat-score variables of the seven habitat characteristics that were measured from each sampling site. NPCA is developed for analysis of categorical data, and may be used for similar purposes as traditional Principal Components Analysis for continuous variables (Norusis 1990). The purpose of this analysis was to create linear combinations of the habitat variables, and to use these linear combinations to describe habitats in further analyses. This method is useful if the resulting linear combinations are well resolved (i.e. statistically valid), and if they give a reasonable biological interpretation. The analysis was conducted using the PRINCAL program, which is included in the SPSS package (Norusis 1990). We used NPCA generated orthogonal habitat-score variables in a standard discriminant analysis to test whether the snail morphs differ in their habitat preferences. Discriminant analysis is used to assess how the groups differ with respect to the independent variables (Norusis 1990). In this case, the habitat-score variables were used to separate the snail morphotypes.

We calculated the prevalence of each of the four cercarial types in each sample for both morphs, and compared the average prevalence of infection by snail morph using Mann-Whitney U-tests. Samples that had no parasites were included in the comparison of prevalence of infection because our purpose was to have a general test for difference in the parasitism rate between the morphotypes. However, we also present results of similar analyses including only the sites where particular parasites were present. Further, we studied the habitat distribution of each parasite type by plotting the 95% confidence intervals of the habitat dimensions for the sites where particular parasites were present. A lack of overlap between these parasite-type-specific confidence ellipses would indicate that particular parasite types are found in specific habitats.
Results

Habitat preference of snail morphs

Univariate analysis suggested that the habitat distribution of the snail morphs differed in type of surrounding habitat and altitude (Table 2). *L. ovata* was found more frequently in natural habitats, surrounded by forests, and *L. peregra* was found more often at higher altitude than *L. ovata*. However, as many habitat variables are strongly correlated, we draw further inferences of the habitat distribution of the snail morphs by using a multivariate analysis.

NPCA yielded two strong habitat dimensions with a biologically meaningful interpretation (Table 3). The position of each original habitat category with respect to these new habitat-score variables is shown in Fig. 2. Altitude was entered in the analysis as a continuous variable, and has similar loading on both habitat dimensions. Therefore, high scores in both habitat dimensions are associated with high altitude. The first habitat dimension separated habitats with shallow running water from those with deeper, standing water. A site with the lowest score for this dimension would be a shallow, low altitude stream with hard substrate. The second habitat dimension separated natural, forest surrounded, more permanent habitats from non-permanent, small agricultural waterbodies. A site with the highest score values for this dimension would have been a small, non-permanent, high altitude pond or stream surrounded by meadows. In short, the first variable was strongly associated with current speed, size, and depth of the habitat (high score values indicating deep habitats without current), while the second variable was associated with the type of surrounding habitat and permanence of the habitat (low score values indicating more permanent habitats in forests, e.g., forest ponds or streams).

Discriminant analysis indicated that these habitat variables sufficiently separated samples by snail morph, especially with the second habitat-score variable (Table 4). Figure 3 illustrates the difference in habitat distribution between the two morphs. The mean position of sites where *L. peregra* was found is clearly shifted upward (Fig. 3), indicating that *L. peregra* was more often found at habitats described by the second habitat dimension (Fig. 3). These results are in accord with the univariate analyses.
Table 2. Results of univariate analyses testing the difference in habitat distribution of the snail morphs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surrounding</td>
<td>$\chi^2 = 6.62$</td>
<td>2</td>
<td>0.037</td>
</tr>
<tr>
<td>Current speed</td>
<td>$\chi^2 = 5.05$</td>
<td>2</td>
<td>0.080</td>
</tr>
<tr>
<td>Shore slope</td>
<td>$\chi^2 = 0.93$</td>
<td>1</td>
<td>0.334</td>
</tr>
<tr>
<td>Altitude</td>
<td>$t = -2.20$</td>
<td>15.98</td>
<td>0.043</td>
</tr>
<tr>
<td>Substrate</td>
<td>$\chi^2 = 3.73$</td>
<td>2</td>
<td>0.155</td>
</tr>
<tr>
<td>Habitat permanence</td>
<td>$\chi^2 = 0.83$</td>
<td>2</td>
<td>0.400</td>
</tr>
<tr>
<td>Habitat size</td>
<td>$\chi^2 = 1.11$</td>
<td>1</td>
<td>0.291</td>
</tr>
</tbody>
</table>

Table 3. Results of NPCA using habitat characteristics listed in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Dimension 1</th>
<th>Dimension 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>0.4236</td>
<td>0.2651</td>
</tr>
<tr>
<td>Multiple fit (%) of fit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altitude</td>
<td>52.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Habitat size</td>
<td>58.0</td>
<td>42.1</td>
</tr>
<tr>
<td>Substrate</td>
<td>63.6</td>
<td>36.4</td>
</tr>
<tr>
<td>Current speed</td>
<td>92.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Habitat permanence</td>
<td>67.8</td>
<td>32.2</td>
</tr>
<tr>
<td>Shore slope</td>
<td>86.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Surroundings</td>
<td>23.5</td>
<td>76.5</td>
</tr>
</tbody>
</table>
**Table 4.** Results of Discriminant Analysis where habitat variables were used to separate the two snail morphs. Habitat variables were generated using the NPCA presented in Table 2. Tests of the differences in the position of snail morphs with respect to both habitat variables are presented in the *Habitat dimension rows.*

<table>
<thead>
<tr>
<th></th>
<th>Correct classification of cases</th>
<th>Eigenvalue</th>
<th>Wilks' Λ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discriminant function</td>
<td>63.79%</td>
<td>0.1410</td>
<td>0.876</td>
<td>0.027</td>
</tr>
<tr>
<td>Habitat dimension 1</td>
<td></td>
<td>0.979</td>
<td>0.283</td>
<td></td>
</tr>
<tr>
<td>Habitat dimension 2</td>
<td></td>
<td>0.898</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Position of habitat categories with respect to scores in nonlinear principal components analysis. Altitude was entered as a continuous variable; therefore, the position of each sample is indicated in the graph. Numbers next to altitude symbols refer to altitude in meters. Note that altitude increases diagonally in the graph. Mean (±SE) position of each snail morph is indicated with large gray circles (open L. ovata, closed L. peregra).
Fig. 3. Position of snail morphs with respect to two habitat dimensions. Lines connect position of each sample to the mean. Error bars around the mean position are ± 1 SE.
Prevalence of trematode infections

The prevalence of the four cercarial types by snail morph is presented in Table 5. The overall prevalence of infection was relatively low, and the differences in the total prevalence of infection by snail morph were statistically not significant. However, the morphs differed with respect to monostome cercariae that had higher prevalences of infection in L. ovata. Note that if the analysis is corrected for multiple tests, the difference is not statistically significant. Trematode parasites were not found in 27% of the snail populations; the snail morphotypes did not differ in this respect (Table 5). If the prevalence of infection was compared using only the populations where parasites were present, the result remained qualitatively similar; the morphs did not differ in their infection rates. However, the difference in the prevalence of monostome infections disappeared ($P > 0.05$). Furthermore, graphical analysis suggests that the parasite types did not differ in their habitat distribution (Fig. 4), indicating that habitat characteristics are poor predictors of the parasite types found at a particular site.

Seasonal variation in parasite prevalence may have confounded the difference in the prevalence of infection between the morphs. Our collections spanned over 6 months, from April (13 samples) to September (one sample), the sample size being sufficient to allow constructing monthly comparisons over the first 5 study months. We did not find a statistically significant difference in the overall prevalence of infection among these months suggesting that seasonal variation may not be an important confounding factor (one-way ANOVA, $MS = 41.41$, $F_{4,55} = 0.66$, $P = 0.62$, error $MS = 62.41$).
Fig. 4. Ninety-five percent confidence ellipses drawn using habitat scores of the sites with particular parasites present and those with particular parasites absent. Sites that overlap indicate that sites with particular parasites do not differ with respect to habitat characteristics. The confidence ellipse limits the area within which the group centroid is found with 95% probability. The mean (±95% confidence interval) position of each snail morph is indicated with gray symbols. (open L ovata, closed L. peregra).
Table 5. Prevalence of infection by snail morph, and the proportion of sites where no parasites were found. The difference in the prevalence of infection was tested using Mann-Whitney U-test, and differences in the proportion of sites without any parasites were tested with \( \chi^2 \)-tests. A statistically significant difference between the morphs at 5% risk is indicated if superscript letters are different between the means/proportions. When only populations that had the focal parasite were included in the prevalence-of-infection comparison, none of the parasites showed significant differences between the two morphs. If \( P \)-values are corrected for multiple tests, none of the \( P \)-values reach significance.

<table>
<thead>
<tr>
<th>Morph</th>
<th>Prevalence of infection (mean %)</th>
<th>SE</th>
<th>Sites with no parasites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All parasites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. ovata</td>
<td>5.64(^a)</td>
<td>0.97</td>
<td>27.9(^a)</td>
</tr>
<tr>
<td>L. peregra</td>
<td>11.72(^a)</td>
<td>3.26</td>
<td>26.7(^a)</td>
</tr>
<tr>
<td>Monostomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. ovata</td>
<td>2.12(^a)</td>
<td>0.56</td>
<td>58.1(^a)</td>
</tr>
<tr>
<td>L. peregra</td>
<td>0.35(^b)</td>
<td>0.25</td>
<td>86.7(^b)</td>
</tr>
<tr>
<td>Xiphidocercariae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. ovata</td>
<td>1.51(^a)</td>
<td>0.52</td>
<td>72.1(^a)</td>
</tr>
<tr>
<td>L. peregra</td>
<td>6.68(^a)</td>
<td>2.86</td>
<td>53.3(^a)</td>
</tr>
<tr>
<td>Echinostomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. ovata</td>
<td>0.63(^a)</td>
<td>0.23</td>
<td>81.4(^a)</td>
</tr>
<tr>
<td>L. peregra</td>
<td>3.17(^a)</td>
<td>2.11</td>
<td>73.3(^a)</td>
</tr>
<tr>
<td>Furcouscercariae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. ovata</td>
<td>0.72(^a)</td>
<td>0.24</td>
<td>81.4(^a)</td>
</tr>
<tr>
<td>L. peregra</td>
<td>0.69(^a)</td>
<td>0.48</td>
<td>86.7(^a)</td>
</tr>
</tbody>
</table>
Discussion

In this study we have shown that the two shell morphs of *L. peregra* differ in their habitat distribution, but do not differ in their rate of parasitism. Hence, our results do not support the idea that parasite-mediated habitat preference would play an important role in the possible reproductive isolation of the snail morphs.

NPCA yielded two well-resolved habitat variables that summarize the general characteristics of typical snail habitats. Both variables were biologically meaningful, and associated habitat characteristics that tend to co-occur. For example, hard substrates are often found together with strong current, and temporary water bodies are often surrounded by meadows. The second habitat dimension clearly separated the two snail morphs. This dimension was mainly associated with the surrounding environment of the freshwater habitat, and with permanence and size of the waterbody. *L. ovata* was more common in forest ponds and streams, while *L. peregra* was more common in small waterbodies in a farming environment, often at higher altitude. Therefore, it appears that *L. peregra* is more often found in smaller freshwater habitats that may be prone to occasional drying. The narrow shell opening of *L. peregra*, limiting water loss by evaporation, may make it more resistant to drying. In addition, the narrow shell of *L. peregra* may facilitate crawling into the mud when the water level suddenly drops.

From the present analysis alone, it is not clear whether the snail morphs are environmentally induced, or whether they are “true” reproductively isolated types. We found only two locations with intermediate shell forms, and the few sites where the distinct morphs were found to be sympatric may be explained by continual drift of one of the morphs from adjacent habitats (e.g., upstream). Hence, these data support the view that the snail morphs are geographically isolated and rarely in contact. In a previous study of a rare case where these morphs were found in the same pond, Ward et al. (1997) concluded, on the basis of genetic, behavioral and ecological data, that the morphs “almost certainly” are separate species. Based on results of the case study mentioned above (Ward et al. 1997; Wullschleger and Ward 1998), and on the extensive field survey presented here, we feel relatively safe in concluding that these morphs show clearly distinct habitat distribution, and that the observed morphological variation is unlikely to be solely induced by the environment.

The question then remains as to which environmental factors have been the major force in causing these differences in habitat distribution. Our motivation for this field survey was the hypothesis that parasite pressure may have played a role. One problem
with this hypothesis is the difficulty in assessing how large a difference in the parasitism rate is required to actually drive habitat specialization. Another problem is that assessment of infection risk by using the proportion of infected individuals as a measure is prone to bias if the susceptibility and exposure to specific parasite species varies, and if the susceptibility of the host types to the entire group of parasites varies. The latter is easier to resolve when several host species whose habitat distributions overlap are included in the analysis. If the hosts have a similar prevalence of infection in similar habitats, the prevalence probably reflects the risk of infection reasonably well. In this study we found that *L. ovata* had a higher prevalence of monostomid cercariae than *L. peregra*, but this difference disappeared when the comparison was conducted using only the samples in which monostomes were found (Table 4). Given that monostomes were not very common, the difference in the prevalence of infection may not justify the conclusion that the parasitism rates of the morphs are significantly different. If trematodes were causing a selection pressure leading to the host habitat distribution observed in this study, one would expect to see a large difference between the morphs in susceptibility to common parasites, or to the entire group of parasites.

In conclusion, factors other than parasites seem to determine the habitat distribution of these two snail morphs. The key habitat characteristics that separate the morphs appear to be the type of the surrounding environment, habitat size and habitat permanence. A simple explanation for this may be adaptive morphology that allows *L. peregra* to persist in occasionally drying habitats. *L. peregra* has a narrow shell that may be advantageous under conditions where occasional dry periods force the snails to crawl into the mud. The larger shell opening of *L. ovata* may lead to lower desiccation tolerance and problems in maintaining movement when water level suddenly drops. However, this survey is not sufficient to conclude that these snail morphs are in a process of incipient speciation that is mediated by habitat specialization. Experiments are needed to show that the fitness of the morphs varies by habitat, if the habitat specialization ideas are correct. The overall prevalence of infection was low, suggesting that parasitism may not be a strong selective factor in these habitats. Whatever the strength of selection, however, it is unlikely that parasitism would drive the habitat specialization of the hosts in this system, because parasites were not confined to specific snail habitats.

**Acknowledgements** We thank M. Brown, P. Mutikainen, J. Taskinen and J. Wiehn for comments on the manuscript. This study was funded by Swiss National Science Foundation grant (#3100-046759.96) to JJ.
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2. Life-history divergence between two closely related freshwater snails, *Lymnaea ovata* and *Lymnaea peregra*

(Esther B. Wullschleger and Jukka Jokela)

**Summary**

When different morphotypes are associated with particular microhabitats, it is possible that the habitat-specific morphotype distribution has been driven by divergent natural selection. Divergent selection may lead to genetic differences, assortative mating, and in the extreme case to speciation. Alternatively, an association between microhabitat and morphotype may indicate either adaptive or neutral phenotypic plasticity, or genetic drift in previous allopatry. Adaptive phenotypic plasticity is not predicted to lead to genetic divergence between morphotypes, and therefore does not play an important role for speciation. To reveal whether there are genetic differences between two closely related freshwater snails which differ in habitat preferences, *Lymnaea peregra* and *L. ovata*, we studied life-history divergence and morphological variation in response to laboratory conditions. The taxonomic status of these morphotypes remains under debate, and it is not known whether the types are environmentally induced or genetically determined. We used two separate origins of each snail morph in a laboratory common garden experiment to test whether life-history variation is more pronounced between populations of different morphs than between populations of the same morph, as predicted if genetic divergence rather than phenotypic plasticity explains the morphotype variation. In addition, we compared shell morphology of wild-caught individuals of both morphs to individuals maintained in the laboratory for two generations. Our results indicate that when snail morphs are grown in a common garden (laboratory), several life-history traits (juvenile growth rate, reproductive schedule) indicate non-plastic divergence, supporting the genetic divergence hypothesis. However, both *L. ovata* and *L. peregra* showed considerable morphological plasticity in response to laboratory conditions and converged to a similar shell form in just two laboratory generations. Overall, our results suggest that genetically based life-history divergence between *L. peregra* and *L. ovata* may be pronounced.

**Key words** Life history, ecological divergence, adaptation, freshwater snail, phenotypic plasticity, speciation
Introduction

Freshwater pulmonate snails, which inhabit a variety of freshwater habitats varying from large lakes to small and temporary ponds, commonly express phenotypic variation in shell morphology and life histories (Russell-Hunter 1978). It is often assumed that this phenotypic plasticity is beneficial, and allows development of phenotypes that are best suited for particular environments (West-Eberhard 1989; Via et al. 1995). This expectation is in accord with the general finding that phenotypic plasticity may be responsible for a considerable part of phenotypic variation in fitness-correlated traits (Bradshaw 1965; Stearns et al. 1991; Thompson 1991; Gotthard and Nylin 1995; Via et al. 1995). However, phenotypic plasticity may come with an additional cost (DeWitt 1998; Scheiner and Berrigan 1998), therefore a canalized specialist phenotype may be advantageous when gene flow and migration between the adjacent habitats is restricted, and selection consistently favors particular habitat-specific trait combinations (van Tienderen 1991). Although plasticity in snail life histories and morphology is often reported, few studies have asked if among-population phenotypic variation also has a genetic component (but see, Johannesson and Johannesson 1996; Trussell 2000).

Separating plasticity from the canalized genetic responses is important for arguments about speciation. For example, if the two morphs show habitat preference for the particular habitat where they perform the best, they may ultimately become sufficiently isolated in terms of morphology and life-history traits, and proceed to reproductive incompatibility and speciation. Such a scenario is a starting point for an ecologically mediated speciation process (Schlüter and Nagel 1995; Schlüter 1996; 1998). In particular, theory predicts that ecological divergence may lead to speciation if divergent habitat preferences have evolved coupled with mate choice (Diehl and Bush 1989), and if divergent selection by local conditions is strong relative to gene flow (Rice and Hostert 1993).

Here, we present a system of two freshwater snail morphs of unresolved species status, which has some characteristics of incipient speciation, but where the roles of phenotypic and genotypic variation in habitat-specific morph distribution are not clear. Lymnaea peregra and L. ovata differ in habitat preferences (Wullschleger and Ward 1998; Wullschleger and Jokela 1999), in distribution across habitat types (Wullschleger and Ward 1998; Wullschleger and Jokela 1999), and in shell morphology (e.g. Hubendick 1951). More specifically, Lymnaea peregra which has a narrower shell form occurs more commonly in small and temporary water bodies situated at higher altitude (Wullschleger and
Ward 1998; Wullschleger and Jokela 1999). However, the snail morphs are attacked by
the same predator community (Reynoldson and Pierce 1979; Young and Procter 1986;
Malmquist 1992; Nyström and Perez 1998), are susceptible to same parasite species, and
do not differ in prevalence of trematode infection (Wullschleger and Jokela 1999). There¬
fore, it remains unresolved to what extent selection regimes differ between the preferred
habitats, although habitat-specific distribution suggests that habitat permanence may be
an important factor separating the two snail types ecologically.
In this study, our goal is to assess whether morphological and life-history variation has a
morph-specific genetic component, or whether it is a result of phenotypic plasticity. Our
results from common garden experiments support the view that L. peregra and L. ovata
are genetically diverged, and although phenotypic plasticity is pronounced in shell mor-
phology, these snails may have experienced divergent selection regimes in the past.
Methods

We present the results of two common garden experiments designed to assess among-population variation in life-history traits. The first experiment was conducted with juvenile snails of around 3-4 mm shell length; the purpose was to compare among-population and between-morph variation in juvenile growth rates. The second experiment was conducted with submature snails of around 6 mm shell length; the purpose was to estimate traits related to reproduction and survival. In both experiments, two different types of environment were applied: low water level and high water level. We assumed that low water level may be taken as a cue that a particular habitat may dry out in the future.

Additionally, we present data on shell morphology; we contrast shell morphology of individuals that have been cultured in the laboratory for two generations to morphology of the original wild-caught snails.

Experiment on juvenile growth rate

We established stock populations of wild-caught snails from four populations, two populations of *L. ovata* (Freiweid, 8°48'E 47°22'N; and Hittnau, 8°49'E 47°22'N) and two populations of *L. peregra* (Chälenhof, 8°35'E 47°11'N; and Erlenmoos, 8°47'E 47°10'N). All populations originated from small freshwater bodies (mostly ponds). In two of these habitats, the snails may have experienced rather temporary conditions with a certain risk of desiccation (Chälenhof and Hittnau), although the pond in Hittnau offered access to deeper water. It was not possible to find habitats of equal "permanence" for *L. peregra* and *L. ovata* respectively, as the former almost always inhabited smaller and less deep freshwater habitats, compared to *L. ovata*.

We kept the snails in laboratory stock tanks (two tanks per each origin) and outbred the snails for two generations. To avoid maternal effects, and to allow sufficient outbreeding, we used F2 offspring in all experiments. We moved the F2 hatchlings to experimental containers (19 × 8 × 9 cm) when they reached the length of 3 mm. We set up a total of 32 containers, each with a starting population of five snails. We created two environments by using two water levels (0.5 liter or 1 liter of water in the container). We then raised these juvenile snails in a climate chamber (20 °C, 12:12 h dark:light period) for ten weeks. The snails were fed *ad libitum* with slightly ground lettuce, supplementing the diet every two weeks with flocks of TetraAniMin fish food. We kept the snails in aged tap water, which
had a teaspoon of ground chalk added per 40 liters, and changed the water and food twice a week. Each container was aerated. We measured the shell length of the snails each week to an accuracy of 0.1 mm with manual calipers.

We did not replace the snails that died during this experiment with new individuals to avoid misidentification of the experimental snails. We included only surviving snails in the analysis. We compared the juvenile growth rate of snail morphs using a repeated measures analysis of variance where population of origin was nested as a random factor below the morphotype. The water level treatment (high vs. low water level) was used as a fixed factor. We used box means of shell length in the analysis. Density varied due to mortality, but adding density in the model as a covariate had no effect on the results. We used three size measures in the repeated measures analysis; size at 3, 6, and 9 weeks after the beginning of the experiment.

Experiment on adult life-history traits

When the F2-generation snails in the stock tanks reached subadult stage (about 6 mm in length), we started an experiment to assess reproductive performance and survival. *L. ovata* and *L. peregra* are known to reach maturity around 7-10 mm (Lam and Calow 1989). The snails we used in this experiment originated from the same field collections as the snails used in the juvenile growth rate experiment (see above), and the same four original populations were represented, with the exception that the *L. peregra* origin of Erlenmoos was replaced with snails originally from a population of Lufingen (8°46’E 47°30’N). We randomly assigned two snails of the same origin to each experimental container. The containers were identical to those used in the juvenile growth rate experiment (see above). We replicated each population of origin 5 times in both high and low water level treatments (40 containers). Laboratory conditions and feeding procedures were identical to the juvenile growth rate experiment (see above). We continued the experiment for 27 weeks.

We used two snails per container to allow social stimulation. Lymnaeids, which are hermaphrodites and capable of self-fertilization, are reported to express low reproductive activity in isolation (Brown 1979; Brown 1983; Vernon 1995); for the same reason dead snails were replaced by slightly smaller conspecifics. We excluded these “companion” snails from all analyses. We measured the shell length once a week, and recorded the number of egg clutches and number of eggs in the clutches twice a week.
We analyzed the differences in mortality between snail morphs using a $\chi^2$-test. We scored mortality in each experimental container as either “no mortality (0)”, “one snail died (1)”, or “both snails died (2)”. The sample size was too low to conduct more detailed analyses by population of origin or by water level treatment. Further, we tested differences in the average survival time (measured in weeks) between morphs, and between water-level treatments using a two-way ANOVA. If both snails had died during the experiment, we used a container mean in the analysis. We included only those containers in the analysis where at least one snail had died.

We analyzed the differences in the proportion of reproductive snails between snail morphotypes, and between water-level treatments using a logit-analysis (McCullagh and Nelder 1989). The analysis was conducted using each container as the experimental unit, i.e. if eggs were found in the container at some point during the experiment, it was scored as “reproductive”. Due to low sample size we had to pool the two origins of the same morph. We tested the effect of morphotype and water level treatment on probability to reproduce by contrasting the fit of logit-models with and without the particular term (analysis of deviance, McCullagh and Nelder 1989; Agresti 1990).

Variation in shell morphology

We compared the aperture length to shell length ratio of individuals that were maintained in the laboratory for two generations to that of wild caught individuals of the same origin. The idea here was to test how shell morphology responds to a change of the environment. The relative aperture length to shell length ratio, also called the relative aperture size ratio (RASR), has been previously used in Lymnaea for taxonomic purposes (e.g. Hubendick 1951). The same analysis using aperture area instead of aperture length did not yield a remarkable difference. Shell width was excluded because of the difficulty to measure this trait on a round shell form. Laboratory stocks were the same as used in the life-history experiments. We tested for differences in shell morphology using a nested analysis of variance, where population of origin was nested under shell morph. The environment (laboratory vs. field) was used as a fixed factor.
Results

Juvenile growth rate

Juvenile growth rate differed significantly between the morphs, while treatment and population of origin within morph had no significant effect (Table 1). Juvenile snails from both L. peregra origins grew faster than snails of the two L. ovata origins, particularly at the subadult stage of around 4.5 to 5 mm (Fig. 1). This result indicates that juvenile growth rate of the snail morphs has a morph-specific genetic component.

Adult life-history traits

There were no differences in the survival of the snail morphs during the experiment ($\chi^2 = 1.75, df = 2, P = 0.417$). One snail died in 16 of the 40 (40%) experimental containers, both snails died in 8 containers (20%), and in 16 containers both snails were still alive at the end of the experiment. Correspondingly, there were no differences in mortality between water level treatments ($\chi^2 = 0.50, df = 2, P = 0.779$). Survival time (measured in weeks) did not differ between the snail morphs, or between water-level treatments (ANOVA: morph, $F_{1,20} = 0.19, P = 0.665$; treatment, $F_{1,20} = 0.68, P = 0.420$, Error $MS = 38.33$), but L. ovata had a shorter survival time in the low-water treatment than in the high-water treatment, while in L. peregra survival time in the low-water treatment was slightly longer than in the high-water treatment (Fig. 2a). However, this interaction effect was only marginally significant (ANOVA: morph × treatment, $F_{1,20} = 4.05, P = 0.058$, Error $MS = 38.33$).

The proportion of containers with reproducing individuals differed clearly between the two snail types, but not by water level treatment (Table 2, Fig. 2b). Only three (15 %) of L. ovata pairs laid eggs during the experiment, while 18 (90 %) of L. peregra pairs reproduced. Similarly, time till the first reproduction was two times longer in reproducing L. ovata (mean ± s.e., 18.67 ± 2.40 weeks) compared to L. peregra (6.7 ± 0.60 weeks; $t$-test, $t = 6.93 df = 19, P < 0.001$). These results suggest that L. peregra and L. ovata populations may have diverged genetically with respect to their reproductive schedule. Total egg production, reproductive rate (eggs/week of reproduction) and clutch size did not differ statistically significantly between the morphs (data not shown). However, the power of the test was very low due to the low sample size in L. ovata ($N = 3$ pairs).
Table 1. Repeated measures analysis of variance comparing the growth rate of juvenile *L. ovata* and *L. peregra* in a laboratory common garden. Two origins (PO) per snail morph (MO) were used. Origin was treated as a random factor and was nested under morph. Three size measurements were used in the analyses: 3, 6 and 9 weeks after the start of the experiments (TI). Treatment (TR) refers to water level treatment; snails were grown either in one liter containers full or half-full of water. Analysis is based on container means. In the beginning each container contained 5 snails, in the end the number of surviving snails varied between 2-5. Superscripts denote error terms for *F*-tests.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between-subjects effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Treatment</td>
<td>0.30</td>
<td>1</td>
<td>0.50a</td>
<td>0.485</td>
</tr>
<tr>
<td>Morph</td>
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<td>1</td>
<td>39.55b</td>
<td>0.024</td>
</tr>
<tr>
<td>Population(MO)b</td>
<td>0.28</td>
<td>2</td>
<td>0.48a</td>
<td>0.627</td>
</tr>
<tr>
<td>TR × MO</td>
<td>0.02</td>
<td>1</td>
<td>0.03a</td>
<td>0.854</td>
</tr>
<tr>
<td>Errora</td>
<td>0.59</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Within-subjects effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>6.40</td>
<td>2</td>
<td>71.89c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TI × TR</td>
<td>0.05</td>
<td>2</td>
<td>0.55c</td>
<td>0.580</td>
</tr>
<tr>
<td>TI × MO</td>
<td>2.45</td>
<td>2</td>
<td>14.88d</td>
<td>0.014</td>
</tr>
<tr>
<td>TI × PO(MO)d</td>
<td>0.16</td>
<td>4</td>
<td>1.85c</td>
<td>0.133</td>
</tr>
<tr>
<td>TI × TR × MO</td>
<td>0.03</td>
<td>2</td>
<td>0.33c</td>
<td>0.719</td>
</tr>
<tr>
<td>Errorc</td>
<td>0.09</td>
<td>52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Juvenile growth rate of two origins of *L. ovata* and *L. peregra* in laboratory containers. The snails used in the experiment were the second laboratory generation of wild caught individuals of four populations, which are indicated by the capital letter in parenthesis after the snail type (C = Chälenhof, E = Erlenmoos, F = Freiweid, H = Hittnau). We measured the snails weekly, the values shown are based on container means (± s.e). We conducted the statistical analyses using the weeks indicated with arrows.
Table 2. Logit-analysis of the proportion of reproductive snail-pairs with respect to morph and water level treatment. Results indicate that snail morph alone is sufficient to explain the variation in proportion of reproducing pairs.

<table>
<thead>
<tr>
<th>Effect</th>
<th>( \chi^2 )</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morph (MO)</td>
<td>27.93</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment (TR)</td>
<td>3.40</td>
<td>2</td>
<td>0.1382</td>
</tr>
<tr>
<td>MO ( \times ) TR</td>
<td>1.20</td>
<td>1</td>
<td>0.2740</td>
</tr>
</tbody>
</table>

Figure 2 (following page). (A.) Survival time and (B.) proportion of adult snails reproducing in a laboratory experiment where *L. ovata* and *L. peregra* snails were kept for 27 weeks in containers of either high or low water level. Values shown are (A.) container means (± s.e), and (B.) frequency of reproducing pairs (± binomial s.e).
capital letters after the snail type (C = Chälenhof, E = Erlenmoos, F = Freiweid, H = Hittnau). Values shown are means (± s.e.). Population of origin is indicated with collected individuals and after two generations in the laboratory (F2-generation) as before (F, Field).

Figure 3: Aperture length to shell length ratio of T. ovata and T. peregra before (F, Field) and after two generations in the laboratory (C, E, F, H).
Variation in shell morphology

Interestingly, the shell morphology of both *L. ovata* and *L. peregra* converged to a similar phenotype in just two laboratory generations (Fig. 3, Table 3). This result indicates that shell morphology responds quickly to environmental change, which largely may be due to phenotypic plasticity, although the design allowed evolutionary response to laboratory conditions. The change in morphology was less for *L. peregra* (Fig. 3), but still statistically significant (ANOVA, including only *L. peregra*: $F_{1,86} = 15.49$, $P < 0.001$).

**Table 3.** Nested analysis of variance testing for morphological response to the laboratory environment in two generations. Factor “environment” (ENV) contrasts wild caught individuals to the laboratory maintained F2-individuals of the same populations. Populations (PO) are nested within snail morphs (MO). Superscripts denote error terms for $F$-tests.

<table>
<thead>
<tr>
<th>Effect</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>2067.73</td>
<td>1</td>
<td>194.17a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morph</td>
<td>831.23</td>
<td>1</td>
<td>3.14b</td>
<td>0.219</td>
</tr>
<tr>
<td>Population(MO)</td>
<td>265.04</td>
<td>2</td>
<td>24.89a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ENV×MO</td>
<td>985.46</td>
<td>1</td>
<td>92.54a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>10.65</td>
<td>26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

We found evidence for genetic divergence between the snail morphs in juvenile growth rate and in reproductive scheduling. *L. ovata* grew slower, started reproduction later, and fewer snail-pairs reproduced during the 27 week experiment than in *L. peregra*. However, laboratory cultured snails of both morphs converged to a similar shell phenotype in two generations. This response is likely to be due to phenotypic plasticity, as such a rapid evolutionary response to selection in the laboratory is unlikely. Hence, the genetic divergence hypothesis was supported by some key life-history traits, while some key morphological traits expressed considerable plasticity.

Previous life-history studies of *L. peregra/ovata* have reported that these snails either follow an iteroparous or a semelparous life-cycle; this seems to depend on the study population (reviewed in Calow 1978). Our results suggest that *L. ovata* follows a more semelparous life-cycle, while *L. peregra* is a continuous breeder. Unclear taxonomic status has probably caused some confusion in the earlier studies. For example, in a study of *L. peregra/ovata* in Lake Zürich, Walter (1977) reported that overwintered *L. peregra* adults lay eggs for few weeks in the spring, after which they die. Based on more recent and extensive surveys, we have good reason to believe that the snails Walter studied were in fact *L. ovata*, which is the most common *Lymnaea* in Lake Zürich (Jokela, personal observation), and follows the reproductive cycle described by Walter (1977). Contrary to Walter’s results, Lam and Calow (1989; 1989) reported, based on a set of detailed field and laboratory studies, that the breeding season of *L. peregra* is 22-26 weeks long. Similarly, *L. peregra* started to reproduce in the 7th week in our experiment, and continued egg laying till the end of the 27 week experiment. Hence, our results suggest that the contrasting results of earlier studies may be explained by a major difference in the reproductive schedule between *L. peregra* and *L. ovata*, suggesting that these two snails follow a fundamentally different genetically determined life-cycle.

If the reproductive schedules of these two snail types are as different as our results indicate, it is possible that these two snail morphs are in fact different species, as suggested by Ward et al. (1997). At the least, such a fundamental difference in the reproductive scheduling would function as effectively as assortative mating in promoting speciation. These snails are rarely found sympatric in the same site (Ward et al. 1997; Wullschleger and Jokela 1999), and attempts to hybridize them in the laboratory have not yet been successful (Wullschleger, unpublished data). Furthermore, evidence for assortative mating has been found in laboratory mate-choice experiments (Wullschleger et al. submitted...
manuscript). Based on this evidence we are tempted to conclude that although the morphs are ecologically similar in many respect (e.g. parasitism, predation, life-span), it may be that they are genetically diverged to the extent that they are different species, or advanced in the process of speciation.

Contrary to reproductive traits, shell morphology of the snail morphs converged to a similar phenotype in the laboratory, which may be taken as evidence against the genetic divergence hypothesis. To our view, this result illuminates how important it is to score as many traits as feasible in studies of genetic divergence. It is not surprising that all traits do not express similar plasticity, and especially if traits express adaptive plasticity, large differences between the traits are to be expected (Bradshaw 1965; Schlichting 1986; Thompson 1991). Shell morphology may indeed depend on several environment-specific factors, for example, habitat type (Crowl 1990; Mukaratirwa et al. 1998), water movement (Lam and Calow 1988; Johannesson et al. 1993), desiccation risk (Vermeij 1971; 1973; Etter 1988), and presence of predators (DeWitt 1998). As indicated by habitat-specific distribution, it is plausible that desiccation risk in temporary habitats and/or strong water movement in more permanent freshwater habitats are important in determining shell form in the lymnaeids presented here, although we did not explicitly address this hypothesis in the present paper.

Adaptive phenotypic plasticity in shell morphology has been found at least with respect to predation (Crowl and Covich 1990; DeWitt 1998), and it has been suggested to be a common response to many other environmental factors as well (Russell-Hunter 1978). This does not contradict the finding of genetic divergence in the reproductive schedule.

In summary, we conclude that evidence is accumulating that Lymnaea ovata and L. peregra are in fact different species. However, species identification based only on shell form may be prone to error due to plasticity in shell morphology. Furthermore, molecular data would be needed to exclude the alternative of genetic divergence by genetic drift as a result of geographic isolation. Further studies should focus on population genetic structure of the snail metapopulations, and genetic distance among versus within the populations of opposing morphotypes. Additional genetic and ecological studies covering wider spatial ranges would also be helpful in attempts to understand the ecological and genetic divergence of these snails.

Acknowledgements
We thank P. Mutikainen and J. Wiehn for comments on earlier versions of the manuscript. This study was funded by Swiss National Science Foundation grant number 3100–46759.96 to J.J.
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Young JO, Procter RM (1986) Are the lake-dwelling leeches, Glossiphonia complanata and Helobdella stagnalis, opportunistic predators on mollusks and do they partition this food resource? Freshwater Biology 16: 561-566
3. Reproductive character displacement between the closely related freshwater snails *Lymnaea peregra* and *L. ovata*

(Esther B. Wullschleger, Jürgen Wiehn and Jukka Jokela)

**Summary**

Theory predicts that enhanced assortative mating, favoring intraspecific matings, should evolve together with reproductive character displacement. Assortative mating may be directly involved in the evolution of species barriers in the case of sympatric speciation, and may strengthen species barriers after secondary contact. If hybrids are at a selective disadvantage, interspecific matings waste reproductive effort and enhanced assortative mating is predicted to be favored. In allopatric populations of the same species, selection for assortative mating is predicted to be weak. Here, we assessed whether sympatric and allopatric populations of the two closely related freshwater snails *Lymnaea peregra* and *L. ovata* mate assortatively. More specifically, we tested populations from several allopatric and one sympatric location for a discrimination against interspecific as compared to intraspecific matings in a series of non-choice mating trials. We found, as predicted by the theory, that snails from the sympatric location avoided to mate with the opposite morphotype, while allopatric snails showed less discrimination against the opposite morphotype. In a broader perspective, our results support the view that reproductive isolation may commonly be reinforced by selection when two closely related taxa occur in sympatry, and that this may also hold for species without any apparent mate signaling traits.

**Keywords:** reproductive character displacement, assortative mating, speciation, *Lymnaea*, reinforcement
**Introduction**

Reproductive isolation between two populations can evolve as a by-product of ecological divergence, or as a response to selection acting directly on reproductive traits (recent reviews include Diehl and Bush, 1989; Schluter and Nagel, 1995; Schluter, 1998; Dieckmann and Doebeli, 1999). If hybrids are at a disadvantage, natural selection on reproductive traits should lead to enhanced avoidance of interspecific matings (assortative mating) in sympatric locations as compared to allopatric locations (Dobzhansky, 1940; Blair, 1955). Individuals mating assortatively would be favored because they avoid mal-adaptive hybridization and waste of reproductive effort (Loftus-Hills and Littlejohn, 1992; Noor, 1999). This process, known as reinforcement of premating isolation, may lead to reproductive character displacement. Reproductive character displacement has been documented on an intraspecific level, and may be involved in the sympatric formation of species barriers (for examples, see Coyne and Orr, 1989; Noor, 1995; Coyne and Orr, 1997; Rundle and Schluter, 1998; but see Ritchie et al., 1992; Gregory et al., 1998).

Most studies on reproductive character displacement involve species or subspecies pairs with highly differentiated mating signals, which allow a concomitant analysis of signaling traits and mate choice. Therefore, taxa with acoustic signaling (Marquez and Bosch, 1997; Castellano et al., 1998), or with more or less complex courtship behavior sequences (Boake, 2000) may be over-represented in this field of research. Here, we use a system of two closely related freshwater pulmonate snails which do not seem to exhibit elaborate mate signaling. In particular, hermaphroditic freshwater pulmonates do not possess ‘female’ mating signals that would enhance the possibility to become fertilized, or ‘male’ mating signals that would enhance receptivity of a ‘female’ (Dillon, 2000). As an aspect of choice, however, ‘females’ can reject sperm donors by displaying rejective behavior, such as shell shaking (Dillon, 2000). Mate choice in pulmonate snails has been reported to depend on the relative sizes of the involved partners (DeWitt, 1996), on parasitic infections (Rupp, 1996), and on whether possible partners originate from sympatric or allopatric populations, suggesting selection favoring the maintenance of local adaptations (Rupp and Woolhouse, 1999).

In our experiments, we investigate whether mating is generally assortative by species, or whether assortative mating is reinforced in sympathy. The first possibility would indicate that the two snail types, or species, are diverged enough to be effectively in reproductive isolation even when allopatric populations get into secondary contact. The second
possibility would indicate that natural selection has reinforced pre-mating isolation in sympatry, thus reducing the fitness costs of hybrid matings.

Our study system does not allow to distinguish whether reproductive isolation in sympatry has been evolving primarily in sympatry, or during secondary contact. In the first case, reproductive character displacement may have been involved in species formation. If significant gene flow between the two snail types is still possible, the first scenario may be more likely. It is not clear if hybridization takes place in natural populations of these snails, but several factors suggest that it is not common. Even if the shell form and life-history traits of these morphs are highly plastic, the morphs have a habitat-specific spatial distribution (Wullschleger and Ward, 1998; Wullschleger and Jokela, 1999), and express genetically based ecological divergence in some life-history traits (Wullschleger and Jokela, submitted manuscript). Moreover, alozyme data on a diagnostic locus suggest no hybridization in our sympatric site (unpublished data). Evidence of ecological and life-history divergence makes it plausible that these snails are in a stage of incipient speciation. Our results support this view by showing that premating isolation is enhanced in sympatry.

Methods

Study organisms

We used the species pair *L. ovata* and *L. peregra* because this system presents a potential example of early divergence and possible incipient speciation. The taxonomic status of these snails has been unresolved up to date, and they are often considered as morphs of one species (Hubendick, 1951; Økland, 1990, *but see* Ward et al., 1997). These snails are simultaneous hermaphrodites and have the capability of self-fertilization. Although they appear to be frequent outcrossers, variation among populations in outcrossing rate can be significant (Jarne and Delay, 1990; Coutellec-Vreto et al., 1997).

Our goal was to test several allopatric and sympatric populations, but extensive sampling (more than 100 locations visited) revealed only one sympatric site with sufficient sample sizes for both snail types (Wullschleger and Jokela, 1999). This site (Seenalpsee, eastern Switzerland) is composed of a shallow, vegetated mud flat which may fall dry
several times during the summer season. Apparently, during high water level *L. ovata* continually migrates into this habitat from a neighboring steep and stony shore, which sustains a stable population (pers. observation Wullschleger).

Mate-choice experiments

We conducted a series of nine non-choice mating trials during the summer season of May to August 1999. We collected the snails for the experiments from 10 allopatric field sites and one sympatric field site in eastern Switzerland. In the first six mate choice experiments, we tested allopatric *L. ovata* and *L. peregra* populations from different sites against each others. In the last three experiments we tested sympatric *L. peregra* and *L. ovata* of Seealpsee against each other (Table 1).

After field collection, the snails were brought to a climate chamber (20°C; 12:12h, dark:light). Prior to the experiments, the snails were kept in isolation in glass jars for about two days. During this time the snails were fed with lettuce *ad libitum*. Individuals which were infected by trematodes and produced cercariae were excluded from the experiment. For the experiments, we placed snail pairs in the wanted combinations into small containers (0.2 liter of water, no food), and left the pairs unobserved for about one hour to familiarize to the new environment and to the offered mate. We arranged both intraspecific (*L. peregra – L. peregra, L. ovata – L. ovata*), and interspecific (*L. peregra – L. ovata*) pairs and observed the matings every 30 minutes for the following five hours. We recorded the occurrence, duration and time of the onset of copulation for each pair. In interspecific pairs, the snail type which acted as a male was also recorded. Each individual was used only once in the experiments and all experiments were conducted during daytime.
Table 1. Non-choice mating trials between allopatric and sympatric *L. peregra* and *L. ovata* individuals. In each experiment both intraspecific (*L. peregra — L. peregra*, *L. ovata — L. ovata*) and interspecific (*L. peregra — L. ovata*) mating combinations were tested simultaneously. Results are presented in Figure 1.

<table>
<thead>
<tr>
<th>Experiments with allopatric populations</th>
<th>Origin of <em>L. peregra</em></th>
<th>Origin of <em>L. ovata</em></th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dietikon 1</td>
<td>Dietikon 2</td>
<td>2-May-99</td>
</tr>
<tr>
<td>2</td>
<td>Lufingen</td>
<td>Freiweid</td>
<td>11-May-99</td>
</tr>
<tr>
<td>3</td>
<td>Länggenbach</td>
<td>Surb</td>
<td>22-May-99</td>
</tr>
<tr>
<td>4</td>
<td>Erlenmoos</td>
<td>Surb</td>
<td>11-Jul-99</td>
</tr>
<tr>
<td>5</td>
<td>Einsiedeln</td>
<td>Volketswil</td>
<td>18-Jul-99</td>
</tr>
<tr>
<td>6</td>
<td>Chälenhof</td>
<td>Surb</td>
<td>25-Jul-99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiments with sympatric populations</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seealpsee</td>
<td>Seealpsee</td>
<td>31-Jul-99</td>
</tr>
<tr>
<td>2*</td>
<td>Seealpsee</td>
<td>Seealpsee</td>
<td>14-Aug-99</td>
</tr>
<tr>
<td>3*</td>
<td>Seealpsee</td>
<td>Seealpsee</td>
<td>18-Aug-99</td>
</tr>
</tbody>
</table>

Note: * indicates the experiments where number of pairs per mating combination was 10. In all other experiments number of pairs used per mating combination was 20.
Statistical analysis

We analyzed the results of the non-choice mating trials using a logit-analysis. We used the frequency of matings observed as response variable, and “Mating Combination” and “Experiment” as factors. We then assessed the significance of each factor by comparing the reduced model (omitting the factor of interest) to a model where this factor was included (McCullagh and Nelder, 1989; Crawley, 1993). The full model contained the main effects of the factors and their interaction. We analyzed the experiments with allopatric and sympatric populations separately. As our primary interest was to compare the mating frequency in the different mating combinations, we further tested each mating combination against others using pairwise contrasts made possible by GENLOG procedure available in SPSS 6.1.1.

We assessed which snail type acted more commonly as a male in matings by using a subsample of interspecific matings in a $\chi^2$-test where the expectation was an equal proportion of matings as a male for both species. Male role can be regarded to indicate which individual initiates mating; ‘male’ individuals attempt mating with no specific courtship behaviors (Dillon, 2000), and copulation starts when ‘females’ accept the attempt.

Furthermore, we analyzed the duration of copulations, contrasting the interspecific copulations to intraspecific ones. We chose a subsample of experiments where a sufficient number of matings in each group was observed, and analyzed the differences in duration of copulation using a two-way mixed-model analysis of variance. Three of the six experiments with allopatric population combinations were included in this analysis. ‘Experiment’ was treated as a random factor, and ‘Mating Combination’ as a fixed factor in the analysis.
Results

Mate choice

Analysis of the allopatric population combinations indicated a strong interaction between the experiment and the mating combination (Table 2). Our prediction was that if assortative mating takes place, the interspecific mating combination would have a lower frequency of matings than either of the intraspecific combinations. In three of the experiments (experiments 1, 4, and 6; Fig. 1) this indeed was the case. However, in the remaining three experiments we observed an intermediate interspecific mating frequency when compared to intraspecific combinations (experiments 2, 3, and 5; Fig. 1). As each of these experiments represents an independent combination of populations (Table 1), this result suggests that evidence for assortative mating between the morphs may be found between some, but not all, allopatric populations.

This result was further clarified when we considered the results of the pairwise contrasts (Table 2). When we contrasted the two intraspecific combinations, the mating frequency was significantly higher in *L. peregra* pairs (Table 2, Fig. 1). Similarly, when we contrasted the *L. peregra* pairs to interspecific pairs, *L. peregra* pairs had a significantly higher mating frequency (Table 2, Fig. 1). However, we found no significant difference in the mating frequency between *L. ovata* pairs and the interspecific pairs (Table 2, Fig. 1). Because the mating frequency of interspecific pairs was not lower than the mating frequency in one of the intraspecific combinations, support for general assortative mating between allopatric snail morphs has to be considered weak.

Analysis of the three experiments that we conducted with the sympatric *L. peregra* and *L. ovata* of Seelalpsee yielded a different result. Here, we found strong support for assortative mating between the snail morphs (Table 3, Fig. 1). Analysis of pairwise contrasts indicated that the intraspecific pairs did not differ in mating frequency, but both intraspecific combinations had a higher mating frequency than observed in the interspecific pairs (Table 3, Fig. 1). In fact, although we found a high frequency of matings in the intraspecific mating combinations, we observed no matings between the two sympatric snail morphs (Fig. 1).

Contrasting the results of these two sets of experiments supports the view that assortative mating between the snail morphs is reinforced in sympathy. Note that even in
the experiments with allopatric populations where assortative mating was supported (experiments 1, 4, and 6; Fig. 1), we always observed some interspecific matings. This is in strict contrast to complete absence of interspecific matings in the sympatric case.

Table 2. Results of logit-analysis for the effect of ‘Experiment’ and ‘Mating Combination’ on the observed mating probability. This analysis includes the six experiments conducted with allopatric *L. peregra* and *L. ovata* populations (Table 1). We further contrasted each of the three mating combinations using pairwise contrasts. Each of the contrasts reports the Generalized Log-Odds Ratio (GLOR), Wald test statistic, and the corresponding significance value. “p-p” refers to *L. peregra* – *L. peregra* pairs, “o-o” refers to *L. ovata* - *L. ovata* pairs, and “p-o” refers to *L. peregra* – *L. ovata* pairs. See Figure 1 for results.

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>$\chi^2$</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating combination (M)</td>
<td>68.04</td>
<td>2</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Experiment (E)</td>
<td>13.04</td>
<td>5</td>
<td>0.02300</td>
</tr>
<tr>
<td>$M \times E$</td>
<td>30.35</td>
<td>10</td>
<td>0.00075</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contrast</th>
<th>GLOR</th>
<th>Wald</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-p vs. o-o</td>
<td>11.66</td>
<td>41.25</td>
</tr>
<tr>
<td>o-o vs. p-o</td>
<td>1.12</td>
<td>0.31</td>
</tr>
<tr>
<td>p-p vs. p-o</td>
<td>12.77</td>
<td>43.48</td>
</tr>
</tbody>
</table>
Table 3. Results of logit-analysis for the effect of “Experiment” and “Mating Combination” on the observed mating probability. This analysis includes the three separate experiments conducted with sympatric *L. peregra* – *L. ovata* population of Seealpsee (Table 1). We further contrasted each three mating combinations using pairwise contrasts as presented in Table 2.

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>$\chi^2$</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating combination (M)</td>
<td>97.12</td>
<td>8</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Experiment (E)</td>
<td>23.85</td>
<td>2</td>
<td>0.00001</td>
</tr>
<tr>
<td>M × E</td>
<td>1.27</td>
<td>4</td>
<td>0.86673</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Contrast</th>
<th>GLOR</th>
<th>Wald</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-p vs. o-o</td>
<td>-0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>o-o vs. p-o</td>
<td>10.34</td>
<td>14.64</td>
</tr>
<tr>
<td>p-p vs. p-o</td>
<td>10.21</td>
<td>13.75</td>
</tr>
</tbody>
</table>
Figure 1. Number of matings observed in no-choice mating experiments with *Lymnaea peregra* and *L. ovata*. (A) In the first set of experiments (Table 1) individuals from one allopatric *L. peregra* and *L. ovata* population were tested in pairs. Different population combination was used in each of the six experiments. (B) In the second set of experiments *L. peregra* and *L. ovata* individuals from sympatric Sealpsee population were tested in pairs. Note that none of the interspecific pairs of sympatric snails mated, although several interspecific matings were observed in experiments conducted with allopatric populations. Values reported are the proportion of pairs mating.
Sexual role and duration of matings

*L. peregra* functioned as a male in 24 (75%) of the 32 interspecific copulations in the dataset ($\chi^2 = 8.00$, df = 1, $P = 0.0047$), suggesting that *L. peregra* was more likely to initiate interspecific matings. Intraspecific *L. peregra* — *L. peregra* copulations lasted significantly longer in all three experiments where sample size was sufficient to allow analysis of copulation duration (Fig. 2). The main effect of mating combination was significant in the analysis of variance (MS = 29.20, $F_{2, 4} = 14.43$, $P = 0.015$; error MS = 2.02), while the main effect of the experiment, or the interaction between the experiment and mating combination were not significant (experiment: MS = 12.23, $F_{2, 88} = 1.80$, $P = 0.171$; interaction: MS = 2.02, $F_{4, 88} = 0.30$, $P = 0.879$; error MS = 6.79). Together, these results suggest that *L. peregra* initiated the interspecific copulations, but their duration was more similar to the duration of intraspecific copulations between two *L. ovata* individuals, i.e. clearly shorter than duration of copulation between two *L. peregra* (Fig. 2).
Figure 2. Duration of mating in three experiments with allopatric *L. peregra* — *L. ovata* populations. Values given are mean (hours) ± 1 s.e. The experiments are numbered as in Table 1 and Figure 1.
Discussion

Our results indicate that assortative mating may occur between the allopatric populations of *L. peregra* and *L. ovata*, but the magnitude of discrimination seems to depend on the identity of the populations tested, and therefore discrimination against the opposite morph cannot be considered as a general rule. The sympatric study site was the only one where we found complete assortative mating, suggesting that pre-mating isolation is considerably enhanced in sympathy, as predicted by the hypothesis of reproductive character displacement.

The results also show differences in mating behavior between the two snail types. In particular, frequency of mating was higher, and copulation duration was longer in the *L. peregra* — *L. peregra* combinations than in the other two mating combinations. This suggests that the snail types differ in the total amount of resources that are allocated to mating, i.e. in their basic reproductive behavior. *L. peregra* functioned as a male in 75% of the interspecific copulations, further supporting the view that the ‘mating drive’ is higher in *L. peregra*. However, the duration of copulations in the interspecific combinations was more similar to the duration of copulations in *L. ovata* — *L. ovata* pairs, suggesting that although *L. peregra* was able to initiate the copulation, it may have lasted less than needed for *L. peregra* to complete the transfer of sperm. Hence, the available evidence points to rather complete reproductive isolation between the snail types. Even if interspecific copulations take place between allopatric snails, we have not been able to find hybrids at the sympatric site, or in laboratory crossings between snail morphs (unpublished data). The fact that sympatric populations between these two snail types appear exceedingly rare (Wullschleger and Jokela, 1999), further suggests that the snail types represent genetically isolated units with a very low ecological opportunity for gene flow. A parsimonious explanation for the enhanced assortative mating in sympathy (i.e. reproductive character displacement) is the waste of reproductive effort caused by the interspecific matings.

Occasional copulations between closely related, but distinct snail species, have been observed in other cases (e.g. Burla and Speich, 1971; Saur, 1990), suggesting that mate choice errors may be common in these organisms with low differentiation of mating signals. For example, *L. peregra*/*ovata* have been found occasionally in copula with a more distantly related species, *L. auricularia*, in Lake Zürich, Switzerland, although intraspecific copulations were overall more common (Burla and Speich, 1971). In that case, no hybrids between the two species were found (Burla and Speich, 1971). These two species apparently have occurred sympatrically in lake Zürich over a longer time period,
which would argue for the evolution of stronger mate choice barriers if interspecific matings were costly. However, *L. auricularia* has been much less abundant as *L. ovata* in Lake Zürich in recent years (Jokela, personal observation), so present selection for mate recognition should be higher in *L. auricularia*. Unfortunately, it is not clear if *L. ovata* or *L. auricularia* functioned more often as a male in the interspecific copulations that Burla and Speich (1971) reported.

A high species specificity in mate discrimination may be expected if efficient conspecific recognition systems have low costs. An interesting example of potential costs of the conspecific recognition system is reported for spadefoot toads. In sympatric populations, female spadefoot toads were found to engage more in species recognition but to be less able to assess male quality, while the females of allopatric populations were less accurate in species recognition but very accurate in recognizing high-quality males (Pfennig, 2000). In our system, mating certainly has fitness costs, for example with respect to loss of energy, loss of foraging opportunities, and increased predation risk (Dillon, 2000). Therefore, the evolution of mate recognition should not easily be constrained by possible costs of the recognition system.

The occurrence of reproductive character displacement in sympatry has been shown in some cases (Marquez and Bosch, 1997; Fishman and Wyatt, 1999), but it also has been reported to be absent in some other potential systems (Veech et al., 1996; Castellano et al., 1998). In this paper, we have reported on a case that supports reproductive character displacement in a system that does not appear to have highly differentiated mating signals (Dillon, 2000). We conclude that although evolution of reproductive character displacement may be more likely in systems where elaborate mating signals are present, our results suggest that this is not a necessary condition for reproductive character displacement to evolve.

**Acknowledgements**

We thank Pia Mutikainen, Paul Ward and Paul Schmid-Hempel for comments on the manuscript. This study was funded by Swiss National Science Foundation grants #31-46759.96 and #31-59242.99 to JJ. JW acknowledges funding from the Academy of Finland.
References


General Discussion and Further Perspectives

The idea that ecological conditions may have some influence on speciation has been prompted by the frequent observation that the rate of speciation varies greatly with ecological circumstances (Schlüter 1998). For example, rapid adaptive radiation commonly occurs in novel, yet uncolonized environments. Such novel environments are thought to provide opportunities to colonize new resource-rich environments in the absence of competitors and/or predators (e.g. Schlüter 1993; Heard and Hauser 1995; Clarke and Johnston 1996). Schlüter (1996b; 1998) proposed several attributes which together indicate that ecological mechanisms may have been causal in speciation: Rapid evolution of assortative mating (cf. Hendry et al. 2000), persistence of two differentiated genotypes in sympathy despite a history of gene flow, a high degree of niche differentiation, and high intrinsic viability and fertility of hybrids. Also, the parallel evolution of reproductive isolation (i.e., parallel speciation) in sister taxa inhabiting similar environments may be indicative of ecological causes of speciation (Dodd 1989; Schlüter and Nagel 1995; Rundle et al. 2000).

Several, though not all, of these attributes are met in the system presented here. Alternative habitat preferences, differentiation in crucial life-history traits and disruptive variation in morphology may indicate a certain degree of niche differentiation. Assortative mating occurred in some, though not all, tested allopatric populations, and appeared to have been reinforced rapidly in a sympatric location, leading to pronounced reproductive character displacement. This sympatric site, Seealpsee, is a small mountain lake which is regulated since the beginning of the 20th century; therefore, its shallow, sympatric shore habitat may be of relatively recent origin. However, the present results do not resolve the question of whether sympatry is of primary or secondary origin in this particular case. This is important because sympatry via secondary contact may also initiate reproductive character displacement between distinct species which do not interbreed, a case in which assortative mating may not have been involved in species formation. In the present case, persistence of the two differentiated morphotypes in sympathy has been confirmed. However, preliminary enzyme analysis revealed no evidence of hybridization in the sympatric site or in laboratory crosses (unpublished data), suggesting that gene flow is, at least, not extensive. Furthermore, there is hypothetical evidence that other groups of freshwater snails show similar tendencies of habitat-dependent segregation of shell types (cf. introduction). In the following, I discuss the obtained results in more detail.
Differing habitat preferences were expressed in a sympatric population and reflected by distributional data from allopatric populations. As shown by the results of my Masters thesis, *L. peregra* seems more tolerant to shallow water and occurs closer to the shoreline on the flat shore at the sympatric site (Seealpsee). This habitat preference may be explained by the hypothesis that the narrower shell form of *L. peregra* restricts water loss and so lowers the risk of desiccation in the shallower zones, which may offer opportunities for colonization. For example, these shallow habitats may offer otherwise untapped food sources, or allow retreat from aquatic predators (e.g., leeches can be escaped by leaving the water, cf. Brönmark and Malmqvist 1986).

In contrast, *L. ovata* tended to avoid the most shallow water depths, but also occurred at a steeper and wave-swept stony shore in Seealpsee, where *L. peregra* was absent. Possibly, strong water movement at the stony shore may prevent colonization by snails with narrow shell forms, such as *L. peregra* from the neighboring sympatric site. Tolerance to strong wave action or current is generally higher in snails with broader shells (Dussart 1987; Trussell 1997). However, as an alternative explanation for the observed pattern, competitive exclusion through the closely related *L. ovata* may have prevented the spread of *L. peregra* into the stony shore habitat at Seealpsee. This alternative has not been investigated. Overall, the distributional data from Seealpsee may suggest that the different habitat preferences of the snails should lead to differences in microdistribution, and, along with this, a certain degree of reproductive isolation, in cases of sympatric occurrence in a water body.

My field survey across the eastern part of Switzerland confirmed that *Lymnaea peregra* is generally more common in smaller, less permanent water bodies and at higher altitude. The correlation between the presence of narrow shell forms of the *Lymnaea peregra-ovata* group and shallow habitats has also been reported by other authors (e.g. Økland 1990). Thereby, intermediate habitat types often supported snails with an intermediate shell form (Økland 1964). These field observations may illustrate that shell form is under the influence of habitat-specific selection pressures. However, the role of phenotypic plasticity in the control of shell form remains unclear in this system.

With respect to biogeography and habitat choice, an important future aspect to investigate would be the question of whether *Lymnaea peregra* is generally more abundant at higher altitudes in European mountain ranges, and whether it is also more abundant in similar habitat types at high latitude. This could resolve the question of whether colonization history or habitat conditions have had more influence in determining the
observed predominance of *L. peregra* at higher altitude in the Swiss Alps. If habitat factors were the stronger force, the ecological speciation hypothesis would gain additional support.

My results in the first chapter also indicate that trematode infections are not important in leading to a spatial isolation of the two snail types. Although the general prevalence of trematode infections varies with respect to habitat factors, this does not correlate with the snails’ distributional differences. If the two snail types would differ greatly in susceptibility to trematode infections, exclusion of susceptible snail types from parasite-rich environments may have been possible, and a correlation of snail type and parasite distribution may have been observed. However, this scenario only reflects the general risk of trematode infection, ignoring that certain trematode species may infect one of the two snail types more commonly than the other. The data show a non-significant tendency for monostome cercariae to occur at a higher prevalence in *L. ovata*. However, monostomes rarely infected these snails, which lowers the possibility that they may be important in the evolution of *Lymnaea peregra* and *L. ovata*. Other parasites of the European molluscan community are considerably less pathogenic than trematodes and may therefore have a minor effect in Lymnaeid evolution. Given that predators of freshwater snails are unlikely to be specific to different snail types (but see Brönmark and Malmqvist 1986; DeWitt et al. 1999), it appears unlikely that parasitism or predation would have been driving the spatial segregation of the two snail types.

If *L. peregra* is generally more common in small and temporary water bodies, as largely confirmed above, life-history differences between the snail types are expected, because temporary ponds, or shores, are much less predictable in the length of breeding season and usually offer lower and less predictable resource levels (Brown 1985a). Shallow, temporary ponds may be considered a habitat with harsh abiotic conditions, a more or less unpredictable dry period obviously being the main factor in limiting colonization by most freshwater organisms (Brönmark and Hansson 1998). In the present case, variation in juvenile growth and in reproductive scheduling differed between the two shell types (Chapter 2). In a common garden experiment, *Lymnaea peregra* grew faster as juveniles, and showed an iteroparous schedule of reproduction. Both these traits may be of advantage in a habitat with unpredictable water level fluctuations, as they allow the rapid re-establishment of populations after a period of drought. Above all, snails in a temporary
habitat may trade-off adult survivorship for high reproductive output (Calow 1981). An iteroparous life-cycle is generally favored under non-permanent habitat conditions with a high risk of unpredictable mortality (Benton and Grant 1999; but see Ranta et al. 2000). Also, survival under desiccation is greater in smaller freshwater snails and in juveniles than in large adults (Storey 1972). Thus, the life-history characteristics of *L. peregra* go along with the theoretical predictions for its preferred habitat type. Most importantly, the two snail types differ clearly in their reproductive schedule, which argues for genetic differentiation in these crucial life-history traits.

In contrast, the shell form of both *L. peregra* and *L. ovata* converged to a similar, narrow shell phenotype in only few laboratory generations. This result indicates that shell morphology can respond quickly to environmental change. It is also in accordance with the observations of an early Lymnaeid taxonomist who bred a number of different shell types of the *L. peregra/ovata* group in the laboratory, and found that their offspring converged to “rather acuminate forms” (Boycott 1938). This response seems to be due to phenotypic plasticity, as such rapid evolutionary responses to laboratory conditions appear unlikely. Since there are several habitat factors which have an influence on shell form, plasticity in shell form may be favored in species which inhabit a relatively broad range of habitats. In the present case, it is of particular interest that different trait groups show different degrees of genetically based divergence. The high plasticity in shell form may indicate that phenotypic plasticity in shell form is important for both snail types. Alternatively, divergent evolution may not have proceeded yet to fixation of this trait.

The results of the mate choice experiments (Chapter 3) indicate that assortative mating has been reinforced to complete isolation between *Lymnaea peregra* and *L. ovata* in a sympatric location, while discrimination against interspecific matings occured only in three of six allopatric population combinations. The first result is indicative of reproductive character displacement, which is predicted to evolve if mismatched matings are costly (e.g. Noor 1999). The second result may illustrate that mate choice in these organisms relies on relatively simple cues, whose underlying traits have not differentiated between the two snail types in all investigated populations. Occasional copulations between closely related but distinct snail species have been observed in other cases (e.g. Burla and Speich 1971; Saur 1990), suggesting that mate choice errors may be common in this group of organisms which have a relatively poor differentiation of mating signals.

In this context, it is surprising that interspecific matings were completely avoided at the sympatric site in Seealpsee. Unfortunately, the potential role of gene flow in the
present system remains unclear. As I found no evidence of extensive hybridisation in the sympatric site (as indicated by a diagnostic locus; unpublished data), it remains unresolved whether reinforcement of premating isolation has been involved in the sympatric formation of species barriers in the past, or whether reproductive isolation has been enhanced when the two snail types met in secondary contact.

The relatively rare occurrence of sympatry of the two snail types (I found only one location in which both snail types were abundant, out of 100 sampled sites) may rather point to the view that the snails are in fact distinct species. If they were in an intermediate stage of divergence, more sympatric populations would be predicted which would also show large variation in the traits that indicate divergence between the two snail types. In the apparently rare case of a sympatric occurrence at Seealpsee, persistence of stable populations of both snail types may be promoted by unique ecological circumstances: As there is an adjacent habitat with different ecological conditions (a steep stony shore), which is inhabited by *L. ovata*, it is possible that the sympatric *L. ovata* population may be continually supplemented by migration from this source population into the sympatric habitat. In the sympatric habitat - a shallow, vegetated mud flat which dries out during the season— *L. peregra* may be at a selective advantage due to its narrower shell form. This could explain the persistence of the sympatric situation in the face of competitive interactions. However, the observation that strong premating barriers evolved in this situation would argue for the view that the role of migration may be minor.

Conclusion and further perspectives

The present study may be considered a first step in deciding whether ecological mechanisms have been initially responsible for the divergent evolution of *Lymnaea peregra* and *L. ovata*. My results also suggest that divergence between these snails is sufficiently advanced to justify species status. However, an extensive molecular genetic analysis would be needed to exclude the possibility of allopatric origin and a non-ecological cause of speciation in this system.

The ecological evidence presented here may suggest that ecological speciation is a likely scenario. In particular, habitat-specific distribution within a largely overlapping range
may be indicative of a non-allopatric species origin. Divergence in life-history traits and disruptive variation in morphology may be interpreted as indicative of divergent niche or habitat use. My data also show that rapid evolution of reproductive character displacement in sympathy has occurred in a present-day population, although assortative mating between allopatric populations is not a general rule. Along with other studies on freshwater snails (Rupp and Woolhouse 1999), this shows that mate choice signals in freshwater snails are sufficiently differentiated to allow genotype-specific assortativeness. Therefore, reinforcement of premating isolation in sympathy may have been possible in this system.

If ecological speciation was affirmed, my results may be of relevance to the understanding of the preconditions, mechanisms, and relative importance of ecological speciation—a major research area in current evolutionary biology. Although a number of case studies are under way, the understanding of ecological speciation still seems to depend on a relatively low number of well-documented cases where the process apparently took place under specific circumstances. Nevertheless, investigations into putative cases of ecological speciation at various stages of divergence may add to the general picture and, in the optimal case, open new lines of research.
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Acknowledgements

I thank Prof. Paul Schmid-Hempel and Jukka Jokela for their excellent supervision and for providing me an opportunity to perform this work largely on my own ideas. Many further inspirations and insights came from Mark Rigby, Mark Brown, Jürgen Wiehn, and other “snails”. Stefano Rezzonico kept my snails well-fed at times of holidays and other breaks. Also the “bumblebees” Boris Bär, Christine Gerloff-Gasser, Pius Korner and the other contemporary students are thanked for their occasional help or advice. Last but not least thanks to Roland Loosli for never losing his patience in face of computer troubles, and to Christine Reber and Rita Jenny for any organizational help facilitating lab and office work.

I also thank Nigel Thew (Neuchâtel), the responsive members of the Molluscanet and Palaeonet mailing lists, and Prof. F. Strauch (Münster, Deutschland) for important, and interesting, information on fossil snails.

My family, my friends and especially Bruno Schättin are thanked for their support, and for making all or part of these years an enjoyable time.
Curriculum vitae

Esther B. Wullschleger

born October 22, 1966
in Zürich, Switzerland

Education

1983-1986 Diplomhandelsschule, Alte Kantonsschule Aarau
1986-1988 Wirtschaftsgymnasium, Alte Kantonsschule Aarau
1988-1993 Study in biology at Universität Zürich, Philosophische Fakultät II
   Major subject: zoology (courses in ecology, ethology and systematics of
   vertebrates)
   Minor subjects: environmental sciences, systematic botany
   Oekologie, Prof. Paul Ward
   Thesis title: Unterschiede in der Habitatwahl bei zwei Gehäusemorphismen
   von Lymnaea peregra (Pulmonata: Basommatophora) im Seepflugsee
1996-2000 Doctoral study, Abt. Experimentelle Oekologie, ETH Zürich, Prof. Paul
   Schmid-Hempel and Dr. Jukka Jokela

Publications in refereed journals

Wullschleger E.B., Ward P.I., 1998: Shell variation and its impact on habitat choice in a
freshwater Lymnaeid snail. Journal of Molluscan Studies 64, 402-404

Wullschleger E.B., Jokela J., 1999: Does habitat-specific variation in trematode infection
risks influence habitat distribution of two closely related freshwater snails? Oecologia
121, 32-38

Papers in preparation

Wullschleger, E.B., Jokela, J., submitted manuscript: Life-history divergence between two
closely related freshwater snails, Lymnaea ovata and Lymnaea peregra

Wullschleger E.B., Jokela J., submitted manuscript: Reproductive character displacement
between the closely related freshwater snails Lymnaea peregra and L. ovata
Oral presentations at scientific meetings

Differences in habitat choice and life history traits in two shell forms of the freshwater snail *Lymnaea peregra*. Zoologia (Symposium der Schweiz. Zoologischen Gesellschaft); Zürich, march 1995

Parasites as selective agents in two host sister taxa: Prevalences of infection in molluscan intermediate hosts in dependence of habitat factors. Zoologia; Geneva, february 1998

Ecological divergence and habitat specialization in two freshwater snails. Evolutionary diversification – an international symposium honoring Murray J. Littlejohn; Columbia/ MO, USA, june 1999

Life-history and morphological adaptation to non-permanent habitats may have caused population divergence between two closely related freshwater Lymnaeid snails. Satellite Symposium of Zoologia & Botanica; Lausanne, february 2000

Poster presentations at scientific meetings

Divergent life-history strategies and incipient reproductive isolation between two freshwater snail morphs: a case of incipient speciation? The Formation of Biodiversity Through Adaptive Speciation – a workshop organized by Ulf Dieckmann, Hans Metz, Michael Doebeli and Diethard Tautz in Laxenburg, Austria; december 10 to 13, 1999

Ecological divergence imposed by habitat permanence and desiccation risk – two freshwater snails on the path to speciation? Phylogeography, Hybridisation and Speciation – a discussion meeting in honour of Godfrey Hewitt; Aussois, France, april 2000

Assortative mating between two closely related freshwater snails is enhanced in a sympatric location. 8th International Behavioral Ecology Congress; Zürich, august 2000

Other important employment positions

1995-1996  Scientific assistant at Zoologisches Museum der Universität Zürich (Prof. P. Ward)

1998-2000  Editorial assistant at Reuters SA, Zürich
Science

"Jede Vermutung ist ein Wagnis – wenn auch der Einsatz nicht gerade das Leben des Wagenden ist, so doch immerhin sein guter Ruf als vernünftiger Mensch und ernster Wissenschaftler. Aber Vermutungen gehören zum Leben der Wissenschaft – so, wie Beweis und Sicherung zum Bestande der Wissenschaft gehören."

Pascual Jordan, 1943. Die Physik und das Geheimnis des organischen Lebens. Die Wissenschaft 95

Life in temporary waters

We have short time to stay, as you,
We have as short a Spring,
As quick a growth to meet decay,
As you or any thing.

Robert Herrick, 1591-1674
Seite Leer /
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