Population genetic structure and mating system evolution in freshwater pulmonates

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Abstract. Freshwater gastropods (Basommatophora and Prosobranchia) harbor a variety of mating systems. In particular, apomictic parthenogenesis in prosobranchs and self-fertilization in the hermaphrodite pulmonates may be viable alternatives to outcrossing sexuality in a number of species. The coexistence of different mating systems in extant populations provides opportunities to examine the forces directing their evolution. We review the models analyzing and predicting genetic variability in subdivided populations, with an emphasis on the effects of inbreeding. Population genetic data on freshwater pulmonates are examined in the context of selfing rates and the loss of variability under selfing. Furthermore, the genetic and demographic factors thought to influence mating system evolution are considered, and we highlight the different approaches available to estimate mating system parameters, in particular the selfing rate. Recent population biological studies on polyploid species (*Bulinus truncatus, Ancylus fluviatilis*) indicate that selfing is the predominant mating system. These studies have contributed to a deeper understanding of conceptual issues in the evolution of selfing rates. Throughout, we emphasize the need for further carefully designed studies.

Key words. Freshwater pulmonates; population genetics; mating systems; selfing; phally; polyploidy; population structure; colonization; allozymes; genetic markers.

"It is relatively easy to show that a given species does or does not possess a capacity for self-fertilization; it is extremely difficult to assess the frequency of selfing in natural populations. This applies to all hermaphroditic organisms, whether they are snails or flowering plants.... For the vast majority of hermaphroditic organisms, therefore, our estimate of the natural frequency of selfing must be either zero or unity or somewhere in between; it will rarely be possible to be more precise."

(Graham Bell 1982)[7]

Introduction

A main concern of population genetics is the distribution of genetic variability within and among populations. This distribution is determined by the joint operation of many factors, e.g. mutation, selection, migration, and genetic drift. Obviously, one important factor is the prevailing mating system, which mediates the way genes are transmitted across generations. This fundamental aspect has been considered quite early by botanists, probably because many plants of agricultural interest are potential selfers. Since the widespread use of protein electrophoresis in population genetics, many studies in plants have indeed analyzed the influence of the mating system on the distribution of genetic variability within and among populations[11,34,103]. In marked contrast, few such studies have been performed in hermaphroditic animals, despite substantial interest among population biologists in estimates derived from organisms other than higher plants (e.g. refs 7, 19).

In a number of prosobranch snails, parthenogenesis rather than selfing is an alternative uniparental mating system with major consequences for the distribution and maintenance of genetic variability. There have been some extensive analyses on this topic in both clonal plants (e.g. refs 8, 27) and animals (e.g. refs 37, 96). Since self-fertilization and parthenogenesis have some similar consequences, and both modes of reproduction occur among freshwater gastropods, we will consider them jointly in the theoretical sections of this paper. However, sound population genetic data on clonal prosobranchs to date are limited (but see refs 26, 53); these circumstances, as well as length limitations, compelled us to restrict our empirical sections to freshwater pulmonates, and hence to an analysis of selfing versus outcrossing.

Another concern of population genetics is the distribution of genes and individuals over time and space. Real organisms may be distributed in a more or less continuous fashion or, alternatively, as fairly small, subdivided populations. Although the same forces operate within and among (sub)populations, it is necessary to consider gene flow, i.e. the movement of genes among populations. Analyzing the consequences of alternative mating systems for finite, subdivided populations is more complex than for 'ideal', infinite populations. However, a recent revival of interest in both the genetics and dynamics of subdivided populations has greatly enhanced our knowledge of the fate of genes under such more realistic circumstances[65,112,113].

Why ought these issues be studied in freshwater snails? First, freshwater snails, and in particular the

hermaphroditic basommatophorans, often occupy both patchily distributed and transient habitats, which are subject to droughts (causing population crashes and even local extinctions) and floods, which may act as important agents of unidirectional migration[67,102,132]. These particular features of their population dynamics make freshwater snails an appropriate group in which to empirically address genetic variability and population genetic structure in subdivided populations.

Second, freshwater snails exhibit a variety of mating systems, including self-fertilization in basommatophorans and apomictic parthenogenesis in prosobranchs[41,42]. It appears likely that both uniparental modes of reproduction evolved repeatedly from an ancestral condition of biparental outcrossing. The coexistence of alternative reproductive modes in certain extant taxa presents exceptional opportunities for causal analyses of the factors involved in mating system evolution (for recent overviews, see refs 68, 116).

Until very recently, the connection between the mating system and population genetic structure in freshwater snails has been largely neglected, despite thorough early work on the mating system considered both at the individual and the population levels in *Bulinus truncatus*[57], and the extensive work of Selander and co-workers in the 1970s on terrestrial gastropods[66,108-110]. In contrast, freshwater pulmonate snails have been largely studied in a biomedical context, mainly because some tropical species serve as intermediate hosts for human schistosome parasites (review in ref. 12). Historically, most of the interest has focused on protein electrophoretic studies performed to discriminate susceptible host (snail) populations and species (e.g. ref. 51).

Our goal is to develop a critical synthesis of 1) how the mating system is expected to influence the distribution of intra- and interpopulational genetic variability, and 2) how the genetic variability in turn may drive the evolution of the mating system. We first consider the models analyzing and predicting the distribution of variability, and emphasize the utility of allozyme data in this regard. We then turn to the relationship between genetic variability and the selfing rate in pulmonates, and discuss polyploidy and aphally as factors in mating system evolution. Finally, we consider the link between mating system and population dynamics. Where appropriate, we will indicate areas in need of further study and the role of molecular (and other) techniques to obtain the critical data.

Population genetic structure in subdivided populations

Snail dispersal and the nature of freshwater habitats

Freshwater snails occupy every kind of freshwater habitat. These habitats are often patchily distributed and can be viewed as islands of freshwater distributed in a 'terrestrial sea'. They may be ponds, lakes, rivers, ditches, or artificial water bodies such as irrigation systems or reservoirs. Even when some of these habitats, such as large lakes, appear to be large and continuous to humans, discontinuity is likely to be imposed on snail populations by environmental factors such as water currents, wave action or food availability, as shown by numerous ecological studies[117,132].

The consequences of habitat patchiness for population genetic structure are expected to depend upon the dispersal abilities of the particular species considered[112]. Most snail species generally disperse little by active means, a counter-example being the upstream crawling of adults in the North American prosobranch *Goniobasis proxima*[21]. However, many gastropods can disperse passively via animal agents such as birds[9] or insects[97], or else during periods of flooding[61,131]. Egg capsules and juvenile individuals can probably also be dispersed when attached to drifting macrophytes or pieces of wood.

Models of subdivided populations

Based on the biology of freshwater pulmonates and the nature of their habitats, it seems reasonable to consider the distribution of genetic variability by using models of subdivided populations (review in ref. 112). We will mainly consider the Island Model and its modified versions (see refs 112, 113, 122), since most predictions on the distribution of genetic variability in subdivided populations have been derived using this model. The original Island Model[133] assumes an infinite number of populations exchanging migrants drawn from a unique pool, to which each population contributes equally at a proportion m. Finite versions of the model have since been derived, in which migrants may originate from only one source population. An alternative model of subdivided populations is the Stepping-Stone Model[55]. In its simplest unidimensional version, populations are regularly distributed along a line or transect. Each population exchanges a fraction $m/2$ of migrants with its two nearest neighbors. The two types of models sometimes behave similarly; we will allude to the Stepping-Stone Model only when necessary.

These models are not intended to describe a geographic reality. Rather, they are a convenient way to handle the distribution of variability, with population size and gene flow between populations being the important parameters[112,113]. For example, it could be more realistic to describe the genetic structure of snails occupying a river using a unidimensional Stepping-Stone Model with unidirectional downstream migration. However, it is possible to use the Island Model and the concept of 'isolation by distance' (i.e. the predicted increased genetic differentiation of populations with increasing geo-

graphic distance[134]) to analyze data in this case, treating an upstream population as a genetic control.

Recent theoretical studies have considered the roles of extinction and colonization processes, inbreeding and the mating system in the Island Model. This is particularly relevant to the study of freshwater snail populations, because the transient nature of their habitats would appear to make population founder events and their concomitant genetic bottlenecks common, and because some freshwater snails can reproduce by (partial) selfing or parthenogenesis as alternatives to outcrossing sexuality[42,49].

Within-population variability

Genetic drift and effective size of populations. Genetic drift is the random variation of allelic frequencies in finite populations that results from the random sampling of gametes for zygote formation (Hartl and Clark[35], chapter 2). Genetic drift results in the fixation or loss of alleles at particular loci and in a decay of heterozygosity. Its effectiveness in eroding variability critically depends on the effective population size, which may be extremely low at times of population crashes or colonization events (population bottlenecks). When the size of a population varies over generations, the magnitude of genetic drift is determined by the harmonic mean of population size (see ref. 35). The magnitude of the loss of both heterozygosity and the number of alleles further depends on the subsequent rate of population growth and the genetic variability before the bottleneck[80]. These considerations are relevant to the study of freshwater snails especially in the tropics, where populations are known to experience dramatic fluctuations in size and where bottlenecks may be common[12,62].

Rare alleles are particularly sensitive to the effects of bottlenecks. The restoration of variability occurs through mutation and/or gene flow, and at equilibrium between genetic drift and mutation, the effective number of alleles is

$$n = 4Nu + 1 = \Theta + 1,$$

and the heterozygosity is

$$H = \frac{\Theta}{\Theta + 1},$$

with N the effective size of the population and u the mutation rate to neutral alleles (ref. 35, p. 124). This highlights that little variability is maintained when N is small.

Influence of the mating system. In an infinite population, selfing leads to the loss of heterozygous genotypes. In other words, the inbreeding coefficient of the population increases (F, the probability that the two alleles at a locus in a given individual are identical by descent). Under the mixed-mating model, allowing a combination of random outcrossing and selfing, and assuming no inbreeding depression, at equilibrium

$$F = \frac{S}{2 - S},$$

with S being the selfing rate. An expected consequence of selfing is heterozygote deficiencies at polymorphic loci, which are indeed commonly found in partially selfing populations (see table 1 for examples in freshwater pulmonates). However, higher proportions of heterozygotes than those expected, based on direct estimates of the selfing rate, are often found in partially selfing species. In other words, outcrossers frequently exhibit slight heterozygote deficits, whereas selfers may not exhibit a deficit as large as expected. This is part of the well-known 'heterozygosity paradox', which has yet to be adequately explained[10,40,63].

Once complete homozygosity has been attained by selfing over many generations, genotypes are transmitted intact across generations when there is no external source of variability (e.g. mutation or gene flow). At that point, selfing and apomictic parthenogenesis have identical genetic consequences. A main difference between the two modes of uniparental reproduction is that heterozygosity, regardless how it was generated initially, can be maintained and replicated across generations via apomictic parthenogenesis, whereas selfing invariably erodes it (for examples, see ref. 42). As we will discuss below, the genetic system of allopolyploids may be viewed as allowing evasion of this diploid paradigm, since the commonly observed 'fixed heterozygosity' allows for the maintenance of biochemical variability irrespective of the selfing rate[1,81,115]. This is analogous to the maintenance of multilocus heterozygosity in diploid parthenogens of hybrid origin.

Loss of variability in finite inbreeding populations. As mentioned above, populations of finite size are expected to gradually loose genetic variability. This effect of finite size is even more pronounced in clonal or selfing populations for at least four reasons.

First, the effective size N_{in} of an inbreeding population is

$$N_{in} = \frac{N_{out}}{1 + F},$$

with N_{out} the effective size of the corresponding random-mating population[93]. The genetic variability is therefore expected to be halved in populations with a very high frequency of self-fertilization. Orive[85] recently showed that the ratio of the effective population size to the census population size is much lower in clonal organisms than in random-mating populations. Whether this will actually promote the loss of variability under natural conditions is not clear, since census population sizes are generally not known for clonal populations.

Table 1. Genetic variability and inferred mating systems in some hermaphrodite freshwater snail species (see text for methods of analysis). With the exception of the tetraploid *Bulinus truncatus*, all species are diploid.

Species	# Pop	# Loci (polym)	# Alleles				Heterozygosity		H-W	Mating system	Reference
			Study		Population						
			polym	all	polym	all	H_{obs}	H_{exp}			
Biomphalaria alexandrina	11	28 (0.21)	2.17 (0.41)	1.25 (0.52)	1.88 (0.19)	1.15 (0.04)	0.051 (0.021)	0.056 (0.022)	2e + 1d (46)	C	Vrijenhoek and Graven[121]
B. camerunensis	12	19 (0.26)	2.20 (0.45)	1.30 (0.58)	1.21 (0.18)	1.06 (0.05)	0.008 (0.012)	0.016 (0.021)	1d (6)	C	Mimpfoundi and Greer[72]
B. glabrata	7	26 (0.15)	2.00 (0.00)	1.15 (0.37)	1.32 (0.28)	1.13 (0.33)	0.000 −0.055	0.021 (0.022)	1e (9)	C	Mulvey and Vrijenhoek[78]
B. glabrata	6	21 (0.57)	2.33 (0.49)	1.76 (0.77)	1.35 (0.12)	1.20 (0.09)	0.017 (0.013)	0.020 (0.015)	0 (36)	C	Mulvey et al.[76]
B. pfeifferi	12	12 (1.00)	2.58 (0.67)	2.58 (0.67)	1.28 (0.15)	1.28 (0.15)	0.007 (0.008)	0.032 (0.030)	6d (7)	S	Bandoni et al.[3]
B. pfeifferi	19	19 (0.21)	2.50 (0.58)	1.32 (0.67)	1.25 (0.16)	1.02 (0.03)	0.001 (0.005)	0.006 (0.013)	3d (4)	S	Mimpfoundi and Greer[73]
B. straminea	4	19 (0.74)	2.80 (0.45)	1.47 (0.84)	2.15 (0.30)	1.35 (0.08)	0.082 (0.018)	0.081 (0.017)	3 (18)	C	Woodruff et al.[130]
Bulinus cernicus	25	6 (0.83)	4.20 (1.48)	3.70 (1.86)	1.44 (0.44)	1.37 (0.43)	0.090 (-)	0.097 (0.091)	0 (27)	C	Rollinson and Wright[101]
B. cernicus	8	11 (0.64)	3.71 (1.25)	2.72 (1.68)	1.82 (0.44)	1.38 (0.54)	0.009 −0.203	0.085 (0.068)	-	C	Rollinson et al.[100]
B. forskalii	10	15 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	0.000 (0.000)	0.000 (0.000)	0	S	Mimpfoundi and Greer[69]
B. forskalii	32	15 (0.20)	2.33 (0.58)	1.26 (0.59)	1.10 (0.08)	1.02 (0.05)	0.001 (0.004)	0.008 (0.016)	3d (3)	S	Mimpfoundi and Greer[71]
B. globosus	15	6 (0.50)	2.67 (1.15)	1.83 (1.17)	1.24 (0.14)	1.12 (0.16)	0.036 (0.056)	0.039 (0.053)	0 (10)	C	Jelnes[51]
B. globosus	13	18 (0.50)	2.89 (1.05)	11.9 (1.21)	1.70 (1.04)	1.52 (0.95)	0.034 (0.019)	0.029 (0.026)	2 (38)	C	Njiokou et al.[84]
B. senegalensis	7	15 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	0.000 (0.000)	0.000 (0.000)	-	S	Mimpfoundi and Greer[69]
B. truncatus	9	14 (0.21)	2.00 (0.00)	1.21 (0.43)	2.00 (0.00)	1.07 (0.27)	0.000 (0.000)	<0.001	1d (1)	S	Jelnes[51]
B. truncatus	13	24 (0.08)	2.50 (0.71)	1.12 (0.45)	1.00 (0.00)	1.00 (0.00)	0.000 (0.000)	0.000 (0.000)	-	S	Mimpfoundi and Greer[70]
B. truncatus	18	42 (0.21)	2.33 (0.71)	1.41 (0.73)	1.04 (0.07)	1.01 (0.03)	0.000 (0.000)	0.005 (0.010)	7d (7)	S	Njiokou et al.[81]
B. umbilicatus	6	7 (0.43)	2.67 (1.15)	1.71 (1.11)	1.56 (0.35)	1.23 (0.36)	0.071 (0.049)	0.079 (0.037)	0 (9)	C	Jelnes[51]
Helisoma anceps	1	26 (0.23)	2.17 (0.41)	1.27 (0.53)	2.17 (0.41)	1.27 (0.53)	0.051 (-)	0.057 (-)	1d (4)	C	Mulvey et al.[75]
Lymnaea auricularia	4	11 (0.36)	2.75 (0.50)	1.64 (0.92)	1.32 (0.17)	1.11 (0.19)	0.037 (0.029)	0.075 (0.057)	6d (12)	M	Coutellec-Vreto et al.[20]
L. elodes	3	15 (0.40)	2.33 (0.52)	1.50 (0.73)	2.22 (0.09)	1.49 (0.04)	0.090 (0.01)	0.113 (0.015)	-	C	Brown and Richardson[13]
L. peregra	11	11 (1.00)	3.36 (1.63)	3.36 (1.63)	2.00 (0.32)	2.00 (0.32)	0.206 (0.200)	0.243 (0.092)	-	C	Coutellec-Vreto et al.[20]
L. peregra	4	12 (0.75)	3.22 (1.79)	2.67 (1.82)	2.56 (0.17)	2.17 (0.12)	0.208 (-)	0.233 (0.020)	-	C	Jarne and Delay[45]
Physa heterostropha	10	10 (1.00)	3.00 (0.94)	2.01 (0.23)	3.00 (0.94)	2.01 (0.23)	0.210 (0.047)	0.260 (0.068)	-	C	Dillon and Wethington (MS)

pop = number of populations studied; # loci (polym) = number of loci studied (proportion of polymorphic loci); # alleles = number of alleles at the scale of the study (study), and averaged over populations (population); 'polym' and 'all' holds for polymorphic loci and all loci, respectively. H_{obs} and H_{exp} are the observed and expected heterozygosities, respectively, averaged over populations. H-W = Hardy-Weinberg equilibrium tested by χ^2; 'e' for excess and 'd' for deficit of heterozygotes with the number of tests performed in parentheses. Mating system: C = predominant cross-fertilization; S = predominant self-fertilization; M = mixed-mating system.

A second reason is that advantageous alleles can drive linked neutral alleles to very high frequencies in selfing populations[38]. This is analogous to what happens when the recombination rate is very low, and has been referred to as 'genetic hitchhiking'. Variability at the presumably neutral marker loci used to analyze genetic variability in selfers can therefore decrease because of genetic hitchhiking. This might also happen in multi-clonal apomictic populations, after a selectively favored mutant is introduced.

The third reason, recently put forward by B. Charlesworth et al.[16], is a form of generalized hitchhiking. Assuming that under a mixed-mating system, some loci are subject to mutation-selection balance and other loci are neutral, these authors showed that only those neutral alleles occurring in gametes bearing the lowest

number of mutations at the selected loci go to fixation. As it does for hitchhiking, the process works at high selfing rates because genotypes do not recombine. B. Charlesworth et al.[16] showed that this process results in the loss of both heterozygosity and number of alleles. This result also holds for clonal populations.

Finally, as already mentioned, small populations are more likely to experience founder effects[4,35]. Selfing populations may be more prone to bottlenecks than outcrossing populations, since they frequently seem to be involved in colonizing situations (see below). The loss of variability in inbreeding populations submitted to bottlenecks has not been analyzed theoretically. For populations of a given size, the absolute loss of alleles is stronger in inbreeding than in outbreeding populations, though the reverse holds for the relative loss of alleles. This is due to the lower effective size of inbreeding populations (P. Jarne, submitted).

Distribution of genetic variability among populations

The distribution of neutral alleles in subdivided sexual populations has been studied by Wright[133], using the infinite Island Model. Wright demonstrated that no significant differentiation is to be expected when $Nm > 1$, with N the effective population size and m the migration rate. When m decreases toward zero, a fraction p of the populations are fixed for the allele of original frequency p. These results also hold for Stepping-Stone Models. An interesting result is that gene flow is related to the genetic differentiation of populations as estimated by F_{st} as follows (F_{st} is the correlation of two randomly chosen alleles in a subpopulation relative to alleles in the whole population[135]):

$$F_{st} = \frac{1}{1 + 4Nm},$$

in the case of two alleles and with $u \ll m$. Slatkin[111,112] and others extended the analysis to the finite Island Model, and also set the results in terms of identity of alleles, either within the same subpopulation or between subpopulations (see Slatkin[112], p. 398). In this case:

$$G_{st} = \frac{1}{1 + 4Nm\alpha} \quad \text{with } \alpha = \left[\frac{n}{n-1}\right]^2,$$

with G_{st} the multi-allelic version of F_{st} defined by Nei[79]. To account for sampling problems, F_{st} must be estimated with the procedure developed by Weir and Cockerham[126]. Models that allow migrants to be recruited at random from only one population, and which consider extinction and recolonization processes, have subsequently been analyzed by Slatkin[111,112] and Wade and McCauley[122]. Results are still set in terms of identity of alleles, and can be compared with the situation under the classical Island Model. These authors showed that F_{st} may increase or decrease, depending on the details of recolonization processes. However, in the propagule

pool model (migrants are drawn from only one population), genetic differentiation is never eroded by local extinction.

The influence of inbreeding upon these models has been analyzed by Whitlock and McCauley[129], Maruyama and Tachida[63], and Jarne (submitted). These authors showed that inbreeding increases the genetic variance among populations and decreases the within-population genetic variance. In some way, inbreeding reduces the effective number of colonists.

In clonal populations, we may expect an interplay of opposing forces: the establishment of new clones by way of migration, mutation, or 'recruitment' from syntopic sexual genotypes[26] being countered by genetic drift, i.e. clone extinction. Maruyama and Kimura[64], considering a multi-allelic haploid model, found that population subdivision and recurrent episodes of extinction and colonization decrease both effective population size and the effective number of alleles (analogous to clones).

Genetic variability in freshwater pulmonates

Most data regarding population genetic variability in freshwater snails have been obtained using starch gel protein electrophoresis. Among the important genetic parameters discussed above, we will focus on the analysis of migration and drift. Selection is neglected here, since allozymes are generally believed to be more or less neutral variants (but see refs 32, 54), and mutation rates are too low to have significant short-term effects. The null model is the Island Model at genetic equilibrium between migration and genetic drift (but see ref. 6 for a comparison of equilibria derived from allozyme versus DNA sequence data).

Methods of data analysis

The species considered here are classified according to their genera, ploidy levels, suspected mating system, geographic range and regional distribution (tropical versus temperate species). Following Hamrick and Godt[34], we consider the following parameters:

Intra-population variability. The parameters are the number of populations, the number of loci and the proportion of polymorphic loci, and the mean number of alleles per locus (and standard deviation) calculated across populations. The expected heterozygosity H_e was calculated for each locus and population as:

$$H_e = 1 - \sum x_i^2,$$

with x_i the frequency of the ith allele. The mean heterozygosity is the average of H_e over populations and loci (with the standard deviation estimated across populations). We also estimated the observed heterozygosity

H_o averaged over populations; this is the value generally given by authors, since genotypic frequencies are not provided in most studies.

Among-population variability. We estimated Nei's[79] genetic diversity statistics H_t and H_s, the total genetic diversity and the mean diversity within populations, respectively, for each locus. G_{st} was then estimated as:

$$G_{st} = \frac{D_{st}}{H_t} = \frac{D_{st}}{H_s + D_{st}}.$$

The standard deviation was calculated across loci.

Results in hermaphrodite freshwater snails

Unfortunately, sampling in most studies has rarely been designed to accommodate thorough population genetic analyses. It is therefore difficult to test for the main factors affecting genetic variability. The roles of genetic drift and bottlenecks may be inferred from the comparison of tropical and temperate genera (table 1), the former generally occupying less stable environments. This may explain the higher variability exhibited by the temperate genus *Lymnaea*, when compared to the two tropical genera, *Biomphalaria* and *Bulinus*. There does not seem to be any obvious relationship between species distribution area and genetic variability. However, the low number of genera studied precludes a meaningful test for any such trend. As no estimate of the effective population size is available, the role of genetic drift in freshwater pulmonates usually can only be suggested, based on their fluctuating distribution in time and space. An exception is a recent study on *Bulinus globosus*[84]. Using a procedure developed by Waples[124], Njiokou et al.[84] showed that under some conditions, the effective size of populations, genetic drift and/or sampling effects can explain the observed variation in allelic frequencies over generations. No genetic data pertaining to local extinctions and recolonizations are available. More convincing is the comparison of selfing and outcrossing species. Of the 24 studies (16 species) listed in table 1, the inferred mating system is selfing in 8 studies (4 species). These species have less polymorphic loci [mean (standard deviation): 19.0 (34.1) versus 53.7% (27.5)], a lower number of alleles per locus [1.05 (0.09) versus 1.41 (0.35)] and a lower expected heterozygosity [0.007 (0.011) versus 0.094 (0.081)] than outcrossing species, as expected on theoretical grounds.

However, the restricted effective population size of selfers alone cannot explain this loss of variability, and environmental effects or even background selection or genetic hitchhiking must be invoked in selfers, which are mostly tropical species. It should also be noted that selfers show consistent heterozygote deficiencies (expected versus observed heterozygosity), compared to Hardy-Weinberg expectations. As expected, they also have higher values of G_{st} [0.575 (0.376) versus 0.318 (0.191)], even when the area sampled per study is taken into account. Finally, it should be noted that the limited genetic variability of most tropical species often precludes drawing firm conclusions about the factors influencing their levels of genetic variability.

Mating system evolution in freshwater pulmonates

The hermaphroditic basommatophorans possess a single gonad called an ovotestis. A potential consequence is the occurrence of self-fertilization, since male and female gametes mature simultaneously and in close

Table 2. Possible advantages and disadvantages of self-fertilization (see text for discussion).

Hypotheses	Advantages	Disadvantages
Genetical	Cost of outcrossing (see text)	Inbreeding depression (see text) Fertility usually lowered in self-fertilizing individuals Male gamete discounting = the reduced production of male gametes with increasing mean selfing rate
	Biparental inbreeding = inbreeding resulting not from selfing but from non-random outcrossing among relatives	
Ecological	Local adaptation = the aptitude of reproducing locally adapted genotypes, *Alternatively*: Maintenance of 'general-purpose genotypes' across generations due to restricted recombination; colonization potential	Variable physical environments = the inaptitude to promote variability or generate diverse genotypes across generations and among offspring to cope with spatial and temporal variability
	Cost of copulation = the many costs involved in finding a partner, courting copulating; exposure to predators	Variable physical environments = the inaptitude to cope with biotic hazards, in particular parasites, which are thought to exert frequency-dependent selection (Red Queen Hypothesis)
	Reproductive assurance = the assurance of reproducing even when isolated	

proximity (for reviews, see refs 25, 31, 49). We are not concerned here with the incidences of transient protandry that have been reported for some basommatophorans (e.g. ref. 128) but emphasize that, once sexually mature, freshwater pulmonates are essentially simultaneous hermaphrodites. While the capacity for selfing has been demonstrated in all species that have been carefully tested, outcrossing appears to be the rule in natural populations[49], although some noteworthy exceptions have been found recently (table 1). This apparent preponderance of outcrossing in the face of possible selfing demands some generally applicable explanation, at the same time illuminating the circumstances under which selfing may be favored over outcrossing (cf. table 2).

In this section, we briefly examine the methods available to estimate the selfing rate, the hypotheses explaining its evolution, and then explore the roles of polyploidy and aphally in *Bulinus truncatus*, traits believed to promote selfing in some hermaphrodite snails. With respect to the available hypotheses for the evolution of selfing in general, we mainly draw on the recent reviews by Uyenoyama et al.[119] and Jarne and Charlesworth[43]. Selfing in pulmonates has been reviewed by Selander and Ochman[110] and Jarne et al.[49].

Estimating the selfing rate

Some special difficulties. The selfing rate can be directly estimated only in experimental settings. Behavioral observations and experiments generally provide few pertinent data, because copulation *per se* is a necessary, but by no means sufficient, event for outcrossing to occur[57,82,115]. Freshwater snails are prone to copulate in normally outcrossing species, particularly after periods of isolation, whereas copulations seem to occur less frequently in selfing species[82]. Nevertheless, copulation may serve functions other than providing allosperm,

such as stimulation of ovulation and/or egg-laying[31]. A second problem occurs in laboratory conditions because the presence of multiple partners can induce lower selfing rates than those occurring in natural populations. A further problem is the more or less continuous production of eggs over the whole life cycle once maturity has been attained, which introduces the possibility of temporal variation in the selfing rate.

Genetic markers and experimental settings. The selfing rate must be estimated using genetic markers. Ideal markers would be neutral variants with codominant, Mendelian inheritance. They ought to be usable at all stages of the life cycle, provide data for both laboratory and field populations, and allow analysis of large numbers of individuals. Predictably, no single available marker satisfies all these requirements (see table 3).

Pigment markers have been used in a few outcrossing species (e.g. *Biomphalaria glabrata*, *Bulinus* sp., *Physa heterostropha*). They have been very useful to show that outcrossing actually occurs, and that allosperm can be stored for several months (for review, see ref. 49). However, as phenotypic characters with a genetic basis, pigment markers are likely to be under selective pressure. For instance, normally pigmented and albino laboratory strains of *B. glabrata* differ in their propensity to outcross as males (M. Vianey-Liaud, pers. comm.), which could be interpreted as the outcome of sperm competition. This feature may restrict the utility of pigment markers for estimating selfing rates, hampered further by the recessivity of the trait[23,86], which severely limits its applicability in natural settings.

The use of DNA markers for population biological studies of freshwater snails is still in its infancy. The only result to date has been the demonstration that the selfing rate can, in principle, be estimated by using

Table 3. Genetic markers available to estimate the selfing rate in hermaphrodite freshwater snails.

Marker	Codominance	Neutrality	Stage of life-cycle scorable		Number of scorable, individuals/effort	Number of alleles (per locus and population)
			Embryo/young	Adult		
Pigments	No	No	Yes	Yes	10^5	1–2
Allozymes	Yes	Yes[c]	No[d]	Yes	10^3	1–5
DNA markers						
Multi-locus fingerprints	No[a]	Yes	No	Yes	10^2	$\approx 10^e$
RAPDs	No[b]	Yes	Yes	Yes	$\approx 2 \times 10^2$	$\approx 10^f$
Single-locus micro- and minisatellites	Yes	Yes	Yes	Yes	$\approx 2 \times 10^2$	$1->10$

[a]Codominance must be established via pedigree analysis.
[b]Codominance may be established via subsequent RFLP analysis.
[c]Generally assumed to hold, but exceptions are known (see text).
[d]Städler et al.[115] have developed a technique allowing to analyze hatchlings of *Ancylus fluviatilis*.
[e]Per probe.
[f]Per primer.

minisatellite probes to generate multilocus fingerprints in the planorbid *Bulinus globosus*[46]. However, this technique has yet to be employed for the comprehensive analysis of natural populations. We will briefly address the potential of DNA-based markers in our concluding section.

Allozymes have been the most widely used genetic markers. Two logically and experimentally differing approaches can be followed to estimate the selfing rate:

1) Direct estimation procedures. Parent-offspring analysis (called progeny-array analysis by plant population biologists; see refs 11, 98) involves the comparison of maternal genotype and genotypes of a sample of her offspring; it depends upon the availability of segregating polymorphisms in the source population. The model assumes mixed-mating and furnishes single-locus as well as multi-locus estimates of the selfing rate[99]. Extensions of this model allow consideration of more realistic situations than mixed-mating[11]. In contrast to the voluminous work on plants to estimate outcrossing rates utilizing progeny-array data, such analyses have generally not been used in freshwater snails; rather, authors only tested for pure selfing versus pure outcrossing (e.g. refs 77, 121). However, recent work on a genetically unusually heterogeneous population of *Ancylus fluviatilis* has provided just such estimates of outcrossing rates at the level of individuals, suggesting a low population outcrossing rate, while finding higher rates in some families (Städler et al., unpubl. data).

A partial solution to the problem of low intrapopulation polymorphism has been to cross individuals drawn from populations harboring at least one diagnostic locus, and then scoring the resulting progeny for genotype at the marker locus or loci (e.g. refs 82, 115). A general problem in evaluating estimates obtained in this way is introduced by the very nature of hermaphrodite breeding systems. In the absence of additional data pertaining to the actual selfing rate of source populations, it is difficult to distinguish between mating system effects and reproductive isolation, when a substantial number of selfed offspring are obtained. For example, such a result could reflect genuinely high selfing rates in one or both source populations (see ref. 83). Alternatively, such a result could very well reflect incipient reproductive isolation among populations, due to genetic differentiation or other factors.

Increasing degrees of reproductive isolation with increasing geographic distance have indeed been shown in both freshwater (*Biomphalaria glabrata*)[87,88] and terrestrial pulmonate snails (*Arianta arbustorum*)[5], although known counterexamples invalidate the generality of this notion (see ref. 52, on the terrestrial pulmonate *Cepaea nemoralis*). Clearly, these ambiguities in self-fertile hermaphrodites strongly argue for independent esti-

mates of the mating system, as can be provided by the analysis of population genetic structure or studies on the magnitude of inbreeding depression (see below). Furthermore, crossing experiments should be performed using individuals sampled very recently from natural populations to minimize induced variation in the selfing rate due to laboratory conditions.

2) Indirect estimation procedures. As already mentioned, S can be inferred from the inbreeding coefficient (F_{is}), assuming mixed-mating and genetic equilibrium. However, positive values of F_{is} (i.e. a deficiency of heterozygotes) may reflect factors other than selfing, e.g. temporal or spatial variance in allele frequencies (Wahlund Effect)[35], or biparental inbreeding. This would seem to be a general drawback of this indirect approach (for discussion, see ref. 30). However, these other factors are less likely to confound estimation of the mating system as the selfing rate increases.

On the other hand, data on population genetic structure have the inherent advantage over single-generation estimates of potentially reflecting selfing rates averaged over several generations, unless this parameter fluctuates widely across generations. Positive F_{is} values have been the main argument in favor of partial selfing in some populations of freshwater pulmonates (see table 1). In many species, low levels of polymorphism have prevented calculation of F_{is} values, and hence, estimates of the selfing rate. On the other hand, many studies uncovering genetic polymorphism have not been cast in terms of mating system analysis, and low sample size and/or suboptimal sampling design diminish their potential to contribute to our data base.

Some insight into the mating system of populations can be gained from the magnitude of inbreeding depression (δ), since large values of δ are expected in outcrossing species (general review of theory and data in ref. 17). The only studies so far combining independent approaches to estimate the selfing rate in freshwater pulmonates have revealed moderate heterozygote deficiencies and large inbreeding depression in *Lymnaea peregra*[44,45] and *Bulinus globosus*[48], suggesting that these populations reproduce predominantly by outcrossing.

Careful laboratory analyses indicate that selfing is possible in all species studied[49]. Unfortunately, these studies have been restricted to very few genera. Without additional data on natural populations and further taxa, it remains unclear whether species with the potential to self-fertilize do so at appreciable frequency under natural conditions. The available data on basommatophoran mating systems, as inferred from studies of population genetic structure, are compiled in table 1. For convenience, we have classified species as either predominantly selfers, predominantly outcrossers, or as possessing a mixed-mating system. However, selfing is

not a feature of entire populations or species, but rather an attribute of individuals. Hence, variation in the selfing rate may be expected among individuals and, by extension, among populations within species. This expectation has recently been confirmed empirically for *Bulinus globosus*[84] and *Ancylus fluviatilis*[115] (Städler et al., unpubl. data).

The forces shaping the mating system
The genetic consequences of selfing in diploids include the loss of heterozygosity and the partitioning of genetic variability into different homozygous lines. Selfing also leads to the purging of recessive and partially recessive deleterious alleles[17,56]. It is also known that outcrossed offspring tend to be more different from their parents than selfed offspring and that outcrossed offspring tend to differ more among each other than selfed offspring, although the genetic variance among selfed offspring can, under restricted circumstances, in fact be higher than among outcrossed offspring[74]. Naturally, the magnitude of these effects depends on the overall population variability. Finally, outcrossing favors the production of new variants through recombination.

These consequences constitute the basis for the hypotheses that have been devised to explain the evolution of selfing versus outcrossing. More than half a century ago, Fisher[28] pointed out that, all else being equal, a selfing mutant occurring in an outcrossing population would have an immediate fitness advantage, because it can transmit genes by selfing but also by outcrossing as a male. Therefore it has a 50% genetic advantage that is known as the cost of outcrossing[15]. Naturally, this advantage may disappear when selfed offspring have lower fitness than outcrossed progeny. If we define inbreeding depression as

$$\delta = 1 - \frac{W_s}{W_o},$$

with W_s and W_o denoting the fitness of selfed and outcrossed offspring, respectively, then $\delta = 0.5$ is the value at which the cost of outcrossing is exactly compensated. This approach assumes that both the selfing rate and inbreeding depression are fixed entities. However, the mating system may be expected to evolve with the population structure over generations[43,119]. Recent approaches therefore specify the assumed genetic basis of inbreeding depression, which has been explained mainly in two ways, 1) the superiority of heterozygotes over homozygotes or, 2) the maintenance of recessive or partially recessive deleterious mutations in outbreeding populations[17].

This more appropriate, dynamic view of the problem indicates that $\delta = 0.5$ cannot be taken as an absolute rule in predicting the evolution of selfing rates. High inbreeding depression is still expected to be manifest in outcrossing species, as opposed to fairly low inbreeding depression in mostly selfing species. Certainly, the cost

of outcrossing and inbreeding depression should not be portrayed as the only relevant forces at work (see table 2). For instance, selfing could be favored under local adaptation, because selfing reproduces particular multilocus genotypes (whether locally adapted or not) more faithfully.

Although the forces involved in the evolution of the selfing rate are thought to be well understood, their relative importance in freshwater pulmonates remains unclear. The few available data are reviewed in Jarne et al.[49]. Pertinent data, such as estimates of inbreeding depression, are scarce, especially in comparison to the huge data base for plants. Jarne et al.[48] showed substantial inbreeding depression in *Bulinus globosus*. Indirect evidence for the role of founder effects is available from population genetic studies (table 1), emphasizing the potential role of reproductive assurance. Biparental inbreeding likely plays a role in unstable and small populations. However, no estimates of such inbreeding are presently available. Likewise, local adaptation has not been considered (see ref. 125 for studies in angiosperms). However, as snails are not strictly sessile organisms, the spatial scale at which local adaptation (if any) may be anticipated is larger than in plants. The only relevant work is Dillon's elegant study on the prosobranch *Goniobasis proxima*[21,22]. Artificial introductions with genetically marked individuals failed to indicate any local adaptation in these highly subdivided populations, and even suggested an advantage for introduced snails, which partly displaced the resident population. Schrag et al.[104,105] suggested that the Red Queen Hypothesis may partly explain the maintenance of euphally (outcrossing ability) in *Bulinus truncatus* (see contribution by Johnson et al., this issue, pp. 498–509). Finally, the evolution of self-incompatibility systems among freshwater pulmonates cannot be ruled out, since only about 50 species have been studied thus far.

Genetics and mating systems of allopolyploid pulmonates
Little work has been done on snail chromosomes since the 1960s and 1970s (for review see ref. 89). However, it seems well established that polyploidy has evolved repeatedly. All pulmonate polyploids studied genetically appear to be allopolyploids with disomic inheritance[89]. In particular, the tropical planorbid *Bulinus truncatus* is a well-studied example of animal allotetraploidy, for which numerous genetic and ecological data are available (e.g. refs 33, 51, 81, 82). Allopolyploids combine the genomes of two genetically differentiated diploid progenitors, and therefore typically exhibit 'fixed heterozygosity' (=phenotypic or homoeologous heterozygosity)[127] at loci differentiating their ancestors. Disomic inheritance results in the transmission and maintenance of biochemical variability in the absence of segregating, homologous heterozygosity (discussion in ref. 115; see fig. 1).
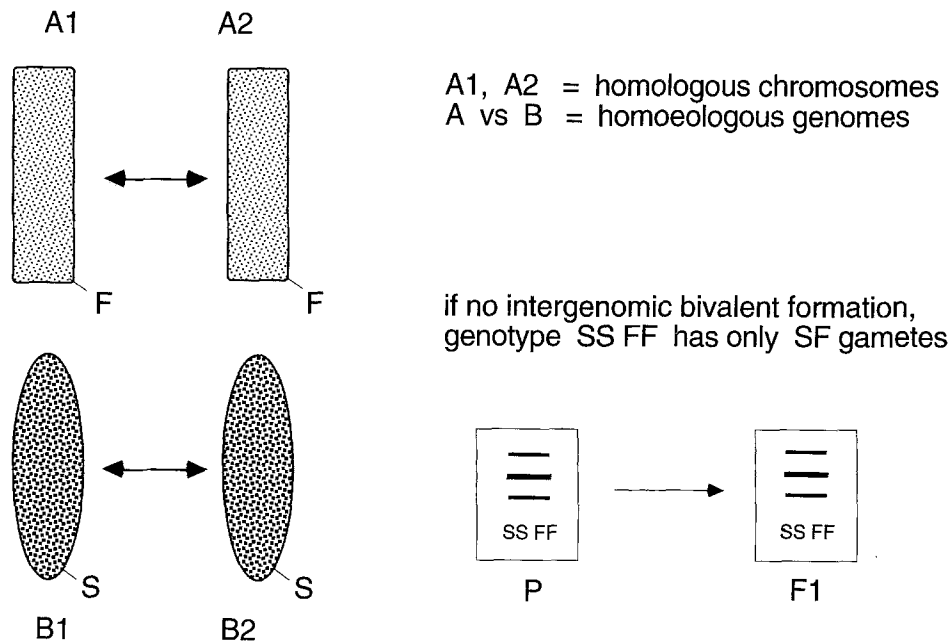
Figure 1. Disomic inheritance and 'fixed heterozygosity' in allotetraploids. The two diploid, ancestral genomes (A and B) are schematically represented by different chromosome shape and stipling. In the allotetraploid, genomes A and B are referred to as homoeologous. The arrows between homologous chromosomes (initially molecular copies of each other; A1-A2, and B1-B2) indicate bivalent formation during meiosis I. Disomic inheritance obtains under strictly *intra*-genomic bivalent pairing, as indicated here. F and S refer to alternative alleles at a random enzyme-encoding locus pair; the expected enzyme phenotypes (zymograms for a dimeric enzyme) for the parental (P) generation and their selfed offspring (F1) are illustrated in the small boxes. For simplicity, it may be assumed that prior to interspecific hybridization, diploid species A was fixed for allele F, and diploid species B was fixed for allele S.

It appears likely that there is a connection between polyploidy, population genetic structure and the mating system, because:

1) The first successful reproductive events after polyploidization are likely to be uniparental, either via selfing or parthenogenesis. Without some propensity for uniparental reproduction, a new polyploid is unlikely to propagate successfully, given the alternative of producing sterile triploid progeny by backcrossing with (presumably more numerous) diploid progenitors.

2) Hybrid origins of new polyploid lineages represent severe genetic bottlenecks, resulting in a purging of the genetic load[39,56]. Continued partial selfing and the sheltering effects of duplicated loci ('fixed heterozygosity') may then set the stage for further selection favoring higher selfing rates. More generally, the genetic load under a mutation-selection model is at least twice as high in diploids as in allotetraploids[56], suggesting favorable conditions for higher selfing rates in allopolyploids. Recently, silencing of duplicate-gene expression ('gene silencing') has been suggested as a potential additional factor influencing the selfing rate of allopolyploids[114,118]. The cause is believed to be homozygosity of non-functional (null) alleles at both loci of a homoeologous pair in the progeny of an outcrossed parent (cf. fig. 1), given that null alleles at both loci segregate in the population. To the extent that a 'null' phenotype at an enzyme locus impairs viability, outcrossing incurs partial sterility among the progeny under such circum-

stances. However, the opposite effect is predicted in the outcrossed offspring, which are thought to experience frequency-dependent selection for lower selfing rates (T. Städler, unpubl. results).

Whether polyploidy is causally related to high selfing rates in freshwater snails remains to be addressed empirically, and more data are certainly needed to draw robust conclusions. The genetics of allopolyploids itself imposes practical constraints in terms of marker availability, at least at the level of protein electrophoresis, since polymorphism (segregating heterozygosity) initially will only be generated by mutation and recurrent origins of polyploid lineages.

Despite these expected constraints, recent allozyme studies of two polyploid taxa have uncovered genetic variability among and within populations. Based on the analysis of population genetic structure, as well as direct estimates of the mating system using interpopulational crosses, the allotetraploids *Bulinus truncatus*[81,82] and *Ancylus fluviatilis*[115] (T. Städler, unpubl. data) both appear to be predominantly selfing. Despite confirmed low outcrossing rates at the population level and many individuals with no detectable outcrossed progeny, recent progeny-array data in *A. fluviatilis* indicate that some individuals exhibit disproportionately high outcrossing rates (Städler et al., unpubl. data).

On the other hand, the tropical diploid planorbids *Bulinus forskalii*[71] and *Biomphalaria pfeifferi*[3] appear to be selfers as well (see table 1). In general, an obligatory

relationship between polyploidy and high selfing rates surely does not hold, since the examples of highly self-fertilizing terrestrial gastropods[66, 108–110] do not involve polyploidy. As suggested by Selander and Hudson[108], the interaction of such ecological factors as low vagility, or patchiness of resources, and particular properties of non-recombining genotypes, may promote high selfing rates, independent of the genetic factors discussed above.

Phally polymorphism in *Bulinus truncatus*

Phally polymorphism is defined as the co-occurrence, in natural populations, of regular hermaphrodite individuals ('euphallics') and individuals lacking the distal part of the male reproductive tract ('aphallics'; see ref. 57). Euphallics can reproduce by outcrossing as male or female, as well as by selfing. Aphallics, however, cannot transmit sperm to mating partners, and reproduce by outcrossing as female or by selfing. As a main consequence of phally polymorphism in *Bulinus truncatus*, an increase in the selfing rate with the proportion of aphallics in the population has usually been assumed[104–107]. This assumption, of course, depends on the euphallics' mating system, which remains to be investigated empirically in sexually polymorphic populations. However, there is currently no evidence from population genetic data for outcrossing among euphallics, and genetic variability at the protein electrophoretic level is as low as in purely aphallic populations[81]. Selfing is the only possibility when all individuals are aphallic.

Aphally ratios vary widely among populations of *B. truncatus*[57,81,104,105] (see fig. 2). Moreover, the determination of aphally versus euphally is still unclear. Genetic components are indicated by Larambergue's[57] and some of Schrag and Rollinson's[106] results, and it is possible to select for higher aphally ratio by mass selection (C. Doums and P. Jarne, unpubl. results). However, nuclear single-gene and cytoplasmic single-gene determination can be ruled out. On the other hand, Schrag et al.[105] and Doums and Jarne (unpublished results) demonstrated that the aphally ratio varies with temperature in some populations, suggesting that the expression of phally is at least partly mediated by local environmental conditions.

Jarne et al.[47] developed a simple model of the evolution of phally polymorphism, with the selfing rate, inbreeding depression and sex allocation as parameters. When the aphally ratio is high, the selfing rate is likely to be high and the inbreeding depression low. Clearly, this model describes only a partial scenario since environmental effects are not accounted for. However, the bias toward high aphally ratios in natural populations (fig. 2) is consistent with the observed low inbreeding depression (C. Doums and P. Jarne, unpubl. results), little if any reallocation of resources toward female function in aphallics[47,106] (Doums and Jarne, unpubl. results), and a high selfing rate of aphallics.
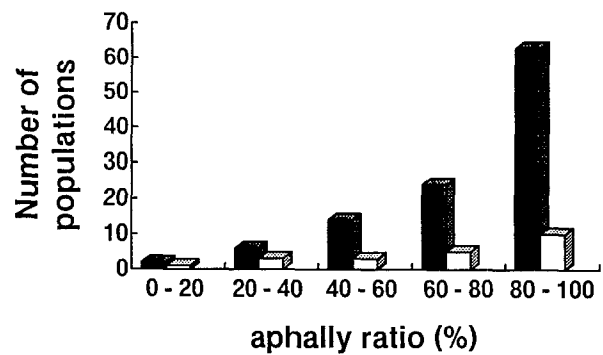
■ Values per population
▢ Mean values per country

Figure 2. Variability of the aphally ratio among natural populations from the whole distribution area of *Bulinus truncatus*, either per country or per population. Data are from Larambergue[57], Njiokou et al.[81], Schrag et al.[104,105], and Mouchet (pers. comm.).

As discussed above, high selfing rates might have a selective advantage under an allopolyploid genetic system. High aphally ratios may therefore be expected in species which can, for whatever initial reasons, be polymorphic for the expression of phally. Selfing decreases the expected genetic variability to extremely low levels, and more quickly than in diploid species. This seems to have occurred in *B. truncatus*, although more variable DNA markers will be required to compare the genetic variability among populations differing in aphally ratios. Ultimately, a tetraploid aphallic population may be able to maintain very high selfing rates, partly due to the permanent hybrid properties associated with 'fixed heterozygosity' and the concomitant evolution of 'general-purpose genotypes' that may be expected under such circumstances (see below).

Mating systems and population dynamics

Population genetics and population dynamics follow the fate of genes and individuals, respectively, over space and time. Mainly for historical reasons, the two fields are rarely considered jointly, an additional reason being that the models tend to become cumbersome. However, knowledge about population dynamics is a prerequisite for the genetic analysis of subdivided populations. Some parameters, such as extinction rates or the rates of increase of populations must be derived from empirical investigations of population dynamics. Population dynamics is most obviously associated with the mating system through density-dependent effects and colonization potential.

Density-dependent effects

The population dynamics of freshwater snails have been studied in natural populations in only a few cases (e.g.

ref. 132). These studies indicate large spatial and temporal variation in density. The connection between density, mating system, and population variability is even less-well understood. However, density may modify the distribution of genetic variability through its action on the mating system for the following reasons:

1) Density modifies the opportunity to mate and therefore to outcross.

2) Inbreeding depression may vary with population density. This has been shown empirically in plants and also holds for *Bulinus truncatus*[24].

3) Density may alter certain life-history traits. For instance, the reproductive output of *B. truncatus* under laboratory conditions is reduced at high densities[24]. Whether reduced reproductive output results in a modification of the mating system, e.g. through an effect on inbreeding depression, has not been analyzed. However, population density correlates neither with the aphally ratio in *B. truncatus*[104,105] nor with the frequency of asexual females in populations of the New Zealand prosobranch *Potamopyrgus antipodarum*[58,59].

4) The parasite load may vary with the local density of intermediate hosts. Whether this reflects greater opportunities for parasite transmission in high-density populations of hosts or may affect the genetic variability of host populations, e.g. through changes in the mating system or differential attack of particular genotypes/clones, is generally unknown. Another possibility is an altered distribution of resistance genes in host populations[29].

The colonization potential

A critical feature of metapopulation models is the (re)colonization rate of 'empty' habitats. Freshwater snails have a high potential for colonization, as exemplified by the rapid colonization of irrigation systems by planorbids in Africa[61], the invasion of the French Antilles by the parthenogenetic prosobranch *Melanoides tuberculata*[91,92], or the rapid spread in Europe of the New Zealand prosobranch *Potamopyrgus antipodarum*[42,94]. Although much remains to be learned about the role of reproductive systems in colonizing species, circumstantial evidence suggests that selfing or parthenogenetic lineages can be particularly successful colonizers.

The prediction has been made that so-called 'general-purpose genotypes'[2] are better at colonizing empty or marginal environments. General-purpose genotypes are characterized by a smaller temporal variance in fitness in fluctuating environments, compared to more specialized genotypes[60]. Because selection can operate on entire multilocus associations of alleles, apomictic parthenogens and highly inbred lineages that persist for considerable time are expected to be tolerant of diverse ecological conditions, which should facilitate successful colonization[95]. On the other hand, an array of clones

that can be continuously augmented from sexual genotypes (and in which individual clones may be short-lived) is more akin to an assemblage of 'frozen' sexual genotypes[26], and not necessarily expected to possess generalist qualities[120].

A general problem for an empirical assessment of the hypothetical relationship between colonizing potential and the mating system is the potential circularity involved, at least for sexual hermaphrodites. For example, it is difficult to discriminate whether high selfing rates are but a consequence of colonization, rather than one of the factors causally related to its initial success. The limited data set in freshwater snails prevents a rigorous test of these alternative hypotheses. We note, however, that the highly selfing *Bulinus truncatus* has a very large geographic distribution. Other outcrossing *Bulinus* species have a large distribution as well, whereas *B. cernicus*, an outcrosser, is endemic to Mauritius[101]. At least at this crude level of analysis, the empirical evidence therefore seems equivocal. For plants, Hamrick and Godt[34] showed that outcrossing is positively correlated with large distribution area. A problem with this kind of correlational evidence is that information on the genetic differentiation over the species' range is generally not available; hence, quite different entities may have been compared. By and large, selfing is probably not a *necessary* precondition for being a good colonizer. Although apomixis has evolved only a few times in freshwater snails[41,42], two apomictic species, *Melanoides tuberculata* and *Potamopyrgus antipodarum*, have extended their distribution in recent years, most likely due to human activities. Ponder[94] argued convincingly for the New Zealand origin of European *P. antipodarum* (= *P. jenkinsi*), the latter having been introduced in the 1850s. In contrast to native New Zealand populations[58,59], most European populations appear to be entirely clonal[123]. A recent DNA fingerprinting study of British populations of *P. antipodarum*[36] documented three widely dispersed, genetically differentiated lineages, attributed to different waves of colonization. The very limited amount of intra-strain polymorphism uncovered by Hauser et al.[36] was thought to reflect mutational divergence since introduction.

Another apomictic prosobranch, *Melanoides tuberculata*, has enjoyed considerable success since its introduction to the French Antilles[90], and may have caused the extinction of indigenous populations of *Biomphalaria glabrata* in parts of Guadeloupe. Moreover, morphologically distinguishable lineages of colonizing *M. tuberculata* differ significantly in life-history features[90]. These patterns are consistent with a model of persistent interclonal selection and the geographical spread of a few generalist genotypes. Recent genetic evidence suggests that at least some clones of *M. tuberculata* are polyploid, and there may be variation in ploidy among clones (B. Delay, unpubl. results). Although it still

seems premature to causally link clonality, polyploidy and colonization potential, these two apomictic prosobranchs appear to be better colonizers, at least in the recent past, than the average prosobranch species.

Conclusions and prospects

As population biologists, we feel that the study of both freshwater pulmonates and prosobranchs has much to offer. However, this potential is only beginning to be adequately appreciated and exploited. For example, the comprehensive research effort to elucidate the genetic consequences and evolutionary significance of alternative reproductive modes among hermaphrodite vascular plants has not yet been complemented by similar efforts in hermaphrodite animals. Conversely, animal parthenogenesis has been thoroughly investigated, particularly in vertebrates and aquatic arthropods (for a recent review, see ref. 14), but relevant studies in gastropods are just beginning. The conceptual and theoretical tools, as well as suitable molecular techniques, are now available to address fundamental issues in evolutionary ecology, taking advantage of the diversity of reproductive strategies realized among freshwater gastropods.

Molecular markers and models of population structure. In empirical studies, sampling design has rarely been adequately considered, although the geographical scale and numerical extent of sampling (at the level of 'populations' and the level of the study) critically affect the kinds of conclusions one may draw. Our analysis indicates that closer attention should be paid to theoretical predictions when planning and evaluating studies on population genetic structure of patchily distributed organisms such as freshwater snails. As previously indicated, protein electrophoresis has not been used to its full potential, partly because many workers were concerned with strain/species identification and other more applied goals. However, where sufficient levels of genetic polymorphism are available, protein electrophoresis remains the technique of choice, based on the kinds of data it yields, its suitability to score large numbers of individuals, and concerns of expense and time (see table 3). Where polymorphism is too low, DNA-based markers have to be employed[46,50]. In either case, robust data sets on population genetic structure of freshwater gastropods have the potential to test theoretical predictions of models for inbreeding and/or polyploid species, and those with limited powers of dispersal.

Evolution of mating systems. Again, the potential of protein electrophoresis to unravel the relationship between the selfing rate and population genetic structure in species exhibiting a reasonable amount of genetic variability (e.g. *Lymnaea peregra*) has not been fully exploited. Furthermore, caution ought to be exercised to avoid circular logic when inferences about selfing rates are based solely on heterozygote deficits, in the absence of other pertinent data. On the other hand, independently derived evidence (direct estimates of the mating system via crossing studies or progeny-array analyses, and estimates of inbreeding depression) will help to explore and clarify the relationship between population genetic structure and mating system parameters. Similarly, we expect that genetic data (allozymes, nuclear DNA markers, mtDNA) will significantly improve our understanding of clonal origins, clonal turnover and clonal richness in parthenogenetic prosobranchs, parameters with important implications for theories on the maintenance of sex (see ref. 26 and contribution by Johnson et al., this issue, pp. 498–509). In addition to population genetic work, life-history studies appear to be very promising, especially in tropical planorbids with easy laboratory maintenance and rapid generation turnover, e.g. some species in the genus *Bulinus*. For example, it seems feasible to monitor the course of self-fertilization depression[48] over several generations in an experimental setting, thus throwing empirical light on the genetic underpinnings of inbreeding depression and the purging of deleterious alleles. Similarly, these species may be utilized to investigate mutation accumulation in experimental inbred populations (see ref. 18). Sex allocation could be studied in relation to the prevailing mating system. Future empirical research on freshwater snails will likely contribute to these and other conceptual issues in population biology.

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