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Mating system, bottlenecks and genetic polymorphism in hermaphroditic animals

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Summary

A loss of neutral genetic polymorphism is theoretically expected for many reasons in inbreeding organisms when compared to outbreeders. The first reason derives from the decrease of the effective population size, down to a halving, in purely selfing species. Other genetical reasons include hitchhiking and background selection. A loss can also be caused by ecological processes, that is by any kind of process provoking a genetic bottleneck. These theoretical expectations have been empirically confirmed in hermaphroditic plants for which selfing species exhibit both a lower gene diversity and number of alleles per population. Here I extend the analysis to hermaphroditic animals, mainly terrestrial and freshwater snails. The decrease of variability in selfers is far greater than what is expected under the halving of the effective size of populations only. Hitchhiking and background selection certainly cannot be rejected as causes of this extra loss. Bottlenecks can clearly be invoked in tropical freshwater snails. However a crude theoretical analysis using Ewens's sampling formulae shows that the relative loss of variability estimated by the number of alleles is smaller in inbreeders than in outbreeders assuming populations with the same number of individuals. This suggests that bottlenecks contribute less to the loss in selfers than in outcrossers. Variability lost within selfing populations of hermaphroditic animals is also lost at the level of a group of populations (metapopulation). This is theoretically not always expected. Indeed, I calculate the ratio of the effective size of a selfing metapopulation to be greater than that of an outcrossing one using previously derived equations. The large variation of this ratio which depends on both migration and effective size of subpopulations prevents prediction of the relative amount of genetic variability stored by selfing and outcrossing metapopulations.

1. Introduction

The history of population genetics is partly confounded with a quest for the conditions in which genetic polymorphism is maintained. Indeed strong forces reduce this below what is expected under ideal conditions, such as high mutation rates, infinite size of populations, random mating among individuals and no selection. I will focus here on two of these forces, namely the mating system (self-fertilization *v.* cross-fertilization) and the restriction of population size (bottlenecks) and consider their influence on presumably neutral polymorphisms (allozymes) in hermaphrodites. Biparental inbreeding will not be considered here as its consequences on polymorphism in selfing hermaphrodites have not been worked out quantitatively.

A well-known consequence of inbreeding is an increase of homozygosity, and the distribution of genetic variability among separate inbred lines (see e.g. Crow & Kimura, 1970). Inbreeding is also known to reduce the genetic variability (see B. Charlesworth, Morgan & Charlesworth, 1993). Kimura & Ohta (1971) showed that a two-allele polymorphism in infinite populations is less able to be maintained at overdominant loci the greater the degree of inbreeding. However, this is not much of concern for our purpose as allozyme polymorphisms are probably not maintained by overdominance (Houle, 1989; David *et al.* submitted). Other reasons for a loss of genetic variability are related to the restriction of the effective population size. First in an inbreeding population the effective population size is expected to be $N_{in} = N_{out}/(1+F)$ with N_{out} the effective size of the corresponding random-mating population and F the inbreeding coefficient at equilibrium (Pollak, 1987). Under the infinite allele model at equilibrium between genetic drift and mutation, gene diversity (Nei, 1987,

chapter 8) is therefore expected to be halved in highly selfing populations provided that the product of the effective population size and the mutation rate is small compared to one. A similar effect on the effective number of alleles is expected when the same product is large compared to one. A second reason is that selectively advantageous alleles can drive linked neutral alleles to very high frequencies in selfing populations (Hedrick, 1980). This is analogous to what happens when the recombination rate is very low, and has been referred to as genetic hitchhiking. Variability at the neutral markers used to analyse the genetic variability in selfers can therefore decrease because of genetic hitchhiking. The third reason, put forward by B. Charlesworth *et al.* (1993), is background selection against deleterious mutations. Assuming that some loci are subject to mutation-selection balance and others 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 are not recombined. Background selection results in a loss of both heterozygosity and the number of alleles. That selfing reduces the genetic variability has found some empirical support. Hamrick & Godt (1989) and Schoen & Brown (1991) using large datasets on plant allozymes showed that indeed the genetic variability in terms of the number of alleles and polymorphic loci as well as gene diversity is far lower in selfers than in outcrossers. However since the overall decline in gene diversity does not exceed 50% it remains unclear whether there can be any role for hitchhiking and background selection. Selander & Ochman (1983) in a more restricted survey also found a reduction in heterozygosity of selfing land snails.

Another major force restricting the genetic polymorphism is population bottlenecks, that is the sudden decrease of effective population size. Bottlenecks may originate from climatic catastrophes such as exceptional droughts for populations of freshwater inhabitants, collapse of pollinator populations for some plants or colonization events. Bottlenecks are important because the effective population size of a population over generations is the harmonic mean of the size at each generation (Crow & Kimura, 1970), and is therefore strongly decreased by any single reduction of the effective size. One must also realize that genetic bottlenecks, that is the reduction of variability *per se*, follows demographic bottlenecks, that is the reduction in the number of individuals, only when the effective size is affected. The consequences of bottlenecks on the number of alleles and gene diversity have been analysed theoretically for random-mating populations, and generally neutral markers (references in Tajima, 1989). Empirical works showed that bottlenecks cause both a loss of alleles and gene diversity (e.g. Leberg, 1992). Barrett & Kohn (1991) and Ellstrand & Elam (1993) have reviewed the relevance of the theoretical results for plant populations. They turned out to be of practical importance for some endangered species.

Theoreticians have addressed the question of the mutation load in small populations worrying about the accumulation of mutations under various conditions (see e.g. D. Charleworth, Morgan & Charlesworth, 1993; Gabriel, Lynch & Bürger, 1993; Hedrick, 1994), and especially when recombination is restricted such as under selfing. However, the consequences of bottlenecks in inbreeding populations on the amount of neutral genetic variability maintained, has neither been worked out theoretically, nor analysed empirically. The question is potentially of importance in endangered selfing plants and in groups as the freshwater snails.

The goal of this paper is to analyse the influence of the mating system and bottlenecks on genetic variability in hermaphroditic animals, extending the previous work of Hamrick & Godt (1989) and Schoen & Brown (1991) in plants and the review of Selander & Ochman (1983) in land snails. The dataset was assembled through a compilation of studies in which both the allozyme variability of natural populations and the mating system are known. Most data are in Pulmonates (Mollusca). Four questions are specifically addressed. (i) Is genetic variability lower in selfers than in outcrossers? (ii) Among freshwater Pulmonates it was also possible to contrast temperate and tropical species, the latter being regularly subjected to population bottlenecks. Do these differences in population size actually depress the genetic variability? (iii) What is the relative influence of mating systems and bottlenecks, and how do they interact in their consequences? (iv) How is the variability distributed at the level of a group of populations?

2. Materials and methods

(i) *Dataset*

Of the 31 animal phyla described (following Adiyodi & Adiyodi, 1983) 21 contain hermaphroditic species (Jarne & Charlesworth, 1993; P. Jarne, unpublished tables). An extensive survey of the literature indicated that data on molecular polymorphisms are available in only 10 phyla, namely Cnidaria, Platyhelminths, Nematoda, Mollusca, Annelida, Arthropoda, Bryozoa, Echinodermata, Tunicata and Vertebrata. However, data for our purpose were required on at least (i) one selfing and one outcrossing species per phylum, (ii) several populations per species, (iii) 20 individuals per sampled population and (iv) several genetic markers per population. (i) conditions the possibility of a comparison. Conditions (ii) to (iv) were retained to have a representative sampling of the genetic diversity per species and decrease the sampling variance. Moreover, a sound knowledge of the reproductive apparatus and a description of the

mating system were necessary. These conditions restricted the analysis to four phyla, i.e. Mollusca, Platyhelminths, Annelida and Vertebrata, and most studies deal with freshwater and land snails (Mollusca–Gastropoda–Pulmonata). Data originated from 73 papers and 55 species. 59 papers describe the genetic polymorphism of snail populations from 43 species. As the only hermaphroditic fish species for which the allozyme variability has been described is the highly selfing *Rivulus marmoratus* I compared this species with gonochoric freshwater fish species chosen among intertropical species.

Selfing does not always result from the mixing of male and female gametes from a single individual ramet or flower. Geitonogamy is defined as selfing occurring among flowers from the same plant, and has an equivalent in colonial animals. Intra-clonal outcrossing is also genetically equivalent to selfing, and occurs in some arthropods such as *Daphnia* for which an extensive dataset on allozyme polymorphism has been gathered (references in Hebert *et al.* 1993). However this will not be considered here as it is difficult to disentangle the genetic consequences of cyclical parthenogenesis and selfing.

Forces besides the mating system and bottlenecks have an influence on or may correlate with the amount of genetic variability. This has been illustrated by Nevo, Beiles & Ben-Schlomo (1984) and Hamrick & Godt (1989) in their surveys: factors such as taxonomic status (e.g. gymnosperms *v.* angiosperms), seed dispersal in plants or life-cycle explain part of the variability uncovered among species. Even if the mating system and the ecology of populations explain a good deal of this variability, removing as much as possible the disturbing factors cited above is necessary, for instance by comparing species within a single taxonomic unit or with similar life-cycles. This was possible when comparing species within land or freshwater snails. However the restricted number of studies in the other groups prevented such a design. Data from selfers on one hand and from outcrossers on the other hand were pooled across phyla.

Data on genetic polymorphism in hermaphroditic animals have been obtained from protein electrophoresis studies, and therefore consist of genic and genotypic individual patterns at the few loci that have been widely studied. Though the idea is still debated (see Gillespie, 1991; Skibinski, Woodwark & Ward, 1993) allozymes will be considered here as neutral markers. The rise of PCR-based technology and sequencing for population studies is too recent to have produced more than preliminary data on variability at the population level. This will be mentioned in the Discussion section.

(ii) *Determining the mating system*

In most studies, the mating system was inferred from behavioural data (copulation or gamete emission observed either in the laboratory or in natural populations) and genotypic datasets. The rationale for this inference is that selfing species are expected to exhibit heterozygote deficiencies, though these latters may arise for many reasons, such as sampling effects (Wahlund effect) or null alleles (Gaffney *et al.* 1990 for review). However heterozygote deficiencies due to other causes than strong inbreeding are unlikely to be of the magnitude observed in this study (see the values of the observed and expected heterozygosities in Appendix) and to occur at all loci as is observed in selfing species. In a restricted number of studies, the mating system was inferred from inbreeding depression estimates or/and progeny-array analyses. In these studies (at least in snails), high inbreeding depression and low selfing rates correlate well with a high propensity to copulate and no heterozygote deficiencies (Jarne, Vianey-Liaud & Delay, 1993). On the other hand, selfing species have low copulation rates compared to outcrossing species. According to the authors of the original studies, species were classified as selfers (S), outcrossers (C) or as reproducing by a mixed-mating system (M). This classification indicates no more than a tendency since intra-specific variation for the selfing rate has been shown in both many plants and some animals (Jarne & Charlesworth, 1993).

(iii) *Parameters and procedures describing the genetic polymorphism*

Data in most studies are given as allelic frequencies at each individual locus. Genotypic distributions are more rarely provided. For each study the following parameters were calculated: number of populations, number of loci, fraction of polymorphic loci out of all loci, mean number of alleles per population for all loci, observed individual heterozygosity H_o averaged over populations, gene diversity H_e (Nei, 1987, chapter 8) as the mean of h_e over loci and over populations with:

$$h_e = 1 - \sum_i x_i^2,$$

where x_i is the frequency of the ith allele at a given locus and population. The standard deviation of H_e was estimated over loci.

G_{ST} (Nei, 1987, chapter 8) is an index of the genetic structure of populations. G_{ST} varies between 0 and 1, and increasing values indicate increased genetic difference among populations. Its standard deviation was estimated over loci. Though other indices of genetic structure are available G_{ST} was retained for comparison with the results of Hamrick & Godt (1989).

It was sometimes not possible to estimate H_e, H_o or G_{ST} from the published data. In three studies (see Appendix 2 and 3), either H_o or H_e was estimated when the fixation index F_{IS} was given by the authors

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using the formulae $F_{IS} = 1 - H_o/H_e$ (Hartl & Clark, 1989, chapter 5). Mean values for the parameters cited above were estimated for freshwater snails, terrestrial snails and other species. Within each group I compared the mean values between selfers and outcrossers (pooling the mixed-maters with outcrossers), and between tropical and temperate species among freshwater snails (*Biomphalaria* and *Bulinus* are tropical genera whereas the three others are temperate genera). For similar comparisons I also calculated in terrestrial and freshwater snails the coefficient of variation for each study of the number of alleles, gene diversity and G_{ST}.

(iv) *The pattern of variation of* N_e

Charkraborty & Neel (1989) developed a procedure for estimating the effective population size N_e. The method assumes the neutrality of the markers used and equilibrium of the populations studied under mutation and genetic drift. This method generalizes to I loci and J populations Ewens's (1972) sampling formulae for the likelihood of observing k alleles in a sample of n genes from a given locus and population. Schoen & Brown (1991) used this procedure to estimate the variation of N_e in selfing and outcrossing plants. Following these authors I assumed that the number of genes sampled in an outcrossing population equals twice the number of individuals whereas it equals the number of individuals in a selfing population. Indeed genes sampled from single individuals are likely to be identical by descent given that in selfing species individuals are highly inbred. As mentioned by Schoen & Brown (1991) it is necessary to assume first an overall mean value for the mutation rate as the mutation rate and the effective population size always appear as a product ($N_e u$). I assumed a mean value of 10^{-5} for u over loci. This of course specifies the absolute values of N_e; however, its pattern of variation is not affected. N_e was estimated using this method for land and freshwater snails using a program provided by D. J. Schoen. The purpose was to obtain patterns of variation in N_e that can be compared to the values given by Schoen & Brown (1991). Mean values and coefficients of variation were compared using Mann–Whitney U-test (Sokal & Rohlf, 1981, chapter 13) as the requirements of the linear model were not met.

3. Results

(i) *Dataset*

The dataset for each species including all the parameters described in the Materials and Methods section (except N_e) and the mating system is given in Appendix 1 (freshwater snails), 2 (land snails) and 3 (other species). Mean values and standard deviations within each group are given in Tables 1, 2 and 3. The observed heterozygosity is sometimes missing. In some species it was also not possible to estimate G_{ST}, generally because there was no polymorphism.

(ii) *Selfers versus outcrossers*

Freshwater snails (Table 1). – 24 studies were analysed. Self-fertilization is the mating system of four species (eight studies). *Lymnaea auricularia* reproduces partially by selfing but genotypic data indicate that the selfing rate is low. This species was therefore pooled with the outcrossers which gives a conservative estimate of the effect of selfing. Selfers and outcrossers do not differ for the number of loci studied though values for selfers were higher. However, they differ for the number of populations studied as well as for all parameters describing the within-population variability (number of alleles and gene diversity): selfers exhibit significantly less variability than outcrossers. The coefficient of variation of the number of alleles and diversity index are higher respectively in outcrossers and selfers. The G_{ST} value of selfers was higher than that of outcrossers though not significantly. G_{ST} may vary with distance among populations. This may bias the analysis if the distance among populations are larger in selfers. A crude test was therefore performed as follows: I calculated Spearman's rank correlation coefficient on log-values of G_{ST} and the largest geographic distance between any populations for each study (this distance has the largest contribution to G_{ST} if G_{ST} correlates with geographic distance). The coefficient does not differ significantly from zero ($r = 0{\cdot}233$, 19 d.f.) indicating that geographic distance has not to be taken into account.

Land snails (Table 2). – Data were analysed on 25 species of which four are selfers, 18 are outcrossers and the remaining reproduces by mixed-mating. Comparisons were performed using selfers *v.* other species and selfers *v.* outcrossers with similar results. No difference was shown for the number of populations and loci studied, and the percentage of polymorphic loci. As for freshwater snails, parameters describing the intra-population variability were higher in outcrossers than in selfers. G_{ST} was significantly lower in outcrossers. As previously no correlation was found between G_{ST} and geographic distance ($r = 0{\cdot}335$, 25 d.f.). In both freshwater and land snails I found no tendency for selfers to exhibit higher coefficients of variation on the number of alleles per locus, the gene diversity and G_{ST}.

Other species (Table 3). – Data are available in 15 species and four phyla only, with one selfing species per phylum. The mixed-mating species *Nereis limnicola* was pooled with selfers. Results were concordant with those found in snails. As the confounding factors cited above may play here our results seem robust. The

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Table 1. *Parameters describing the genetic variability in selfing and outcrossing freshwater snails*

Parameters	Selfers	Outcrossers	U; P
$N_{pop.}$	15·00 (8·03)	8·75 (5·90)	4·35; 0·04
$N_{loc.}$	20·38 (9·34)	15·38 (9·34)	1·09; 0·30
$N_{pol.\,loc.}/N_{loc.}$	0·19 (0·34)	0·54 (0·27)	7·14; 0·007
$N_{all.}$	1·05 (0·09)	1·41 (0·35)	11·37: < 0·001
H_o	0·001 (0·002)	0·084 (0·066)	15·51; < 0·001
H_e	0·007 (0·011)	0·094 (0·081)	13·52; < 0·001
G_{ST}	0·575 (0·376)	0·318 (0·191)	2·80; 0·09

$N_{pop.}$ and $N_{loc.}$ are the number of populations and loci studied respectively, $N_{pol.\,loc.}/N_{loc.}$ is the percentage of polymorphic loci, $N_{all.}$ is the number of alleles per population averaged over all loci, H_o is the individual observed heterozygosity, H_e is the gene diversity and G_{ST} is an index of population structure. Mean values are given with their standard deviations in parentheses. U is the value of the Mann–Whitney test and P its significance. Eight studies on selfers were compared with 16 studies on outcrossers. See text for further details.

Table 2. *Parameters describing the genetic variability in selfing, mixed-mating and outcrossing land snails*

Parameters	Selfers	Mixed-maters	Outcrossers	U; P
$N_{pop.}$	10·60 (7·73)	18·66 (13·20)	11·26 (13·46)	0·57; 0·45
$N_{loc.}$	17·00 (6·60)	12·00 (4·58)	17·35 (7·65)	0·48; 0·49
$N_{pol.\,loc.}/N_{loc.}$	0·32 (0·37)	0·45 (0·49)	0·55 (0·27)	2·83; 0·09
$N_{all.}$	1·19 (0·42)	1·83 (0·88)	1·62 (0·43)	5·92; 0·01
H_o	0·010 (0·022)	0·031 (0·037)	0·097 (0·047)	10·33; 0·001
H_e	0·040 (0·089)	0·130 (0·161)	0·130 (0·084)	4·79; 0·02
G_{ST}	0·775 (0·386)	0·447 (0·039)	0·231 (0·104)	5·88; 0·01

The description of parameters is given in Table 1. The Mann–Whitney U test compares selfers and outcrossers. Five studies on selfers, three on mixed-maters and 23 on outcrossers were considered.

Table 3. *Parameters describing the genetic variability in selfing and outcrossing hermaphroditic animals (snails excluded)*

Parameters	Selfers	Outcrossers	U; P
$N_{pop.}$	5·00 (4·76)	12·10 (9·16)	2·92; 0·09
$N_{loc.}$	18·25 (10·24)	14·20 (6·16)	0·50; 0·48
$N_{pol.\,loc.}/N_{loc.}$	0·22 (0·08)	0·43 (0·24)	7·24; 0·007
$N_{all.}$	1·13 (0·13)	1·59 (0·58)	4·51; 0·03
H_o	0·024 (0·024)	0·148 (0·137)	5·33; 0·02
H_e	0·028 (0·025)	0·129 (0·112)	5·80; 0·01
G_{ST}	0·436 (0·379)	0·210 (0·116)	1·54; 0·21

The description of parameters is given in Table 1. Four selfing species were compared with 11 outcrossing species.

Table 4. *Variation of N_e among snail species. n is the number of studies considered, m is the mean N_e over species with its standard deviation in parentheses and CV is the coefficient of variation averaged over species with its standard deviation in parentheses*

	Freshwater snails			Land snails			All species		
Mating system	*n*	m	CV	*n*	m	CV	*n*	m	CV
Outcrossing	11	4452 (2150)	0·438 (0·269)	6	5705 (1215)	0·342 (0·161)	17	4894 (1932)	0·404 (0·236)
Selfing	3	2834 (498)	0·457 (0·373)	2	6081 (1067)	0·316 (0·222)	5	4133 (1890)	0·401 (0·296)

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Table 5. *Parameters describing the genetic variability in tropical and temperate outcrossing freshwater Pulmonates*

Parameters	Tropical	Temperate	U; P
$N_{pop.}$	10·70 (6·15)	5·50 (4·04)	4·06; 0·04
$N_{loc.}$	16·10 (8·14)	14·17 (6·05)	0·14; 0·71
$N_{pol.\,loc.}/N_{loc.}$	0·48 (0·23)	0·62 (0·34)	0·57; 0·45
$N_{all.}$	1·25 (0·15)	1·68 (0·44)	3·91; 0·05
H_o	0·054 (0·031)	0·134 (0·083)	4·72; 0·03
H_e	0·052 (0·031)	0·163 (0·092)	5·69; 0·02
G_{ST}	0·369 (0·200)	0·169 (0·094)	4·33; 0·04

The description of parameters is given in Table 1. Ten studies of tropical species were compared with six studies of temperate species.

number of populations and loci studied as well as G_{ST} do not differ between selfers and outcrossers. On the other hand the four other parameters are significantly lower in selfers.

Variation of N_e (Table 4). – The studies used for this analysis are given in Appendix 1 and 2. The sample size was fourteen among freshwater snails and eight among land snails. Mean values and their coefficient of variation are given in Table 4. None of the tests performed on either mean values and coefficients of variation rejected the hypothesis of equality between selfers and outcrossers. However it must be noticed that the mean effective size is higher in outcrossers than in selfers among freshwater snails. The important result is that selfers do not exhibit more variation in N_e than outcrossers.

(iii) *Tropical versus temperate freshwater snails* (Table 5)

Values from Appendix 1 were tabulated again so as to contrast tropical and temperate outcrossers. No difference was shown for the number of populations and loci studied and the percentage of polymorphic loci. Values were significantly higher in temperate species for the within-population variability parameters whereas the contrary holds for G_{ST}. The correlation between G_{ST} and geographic distances is still not significant ($r = 0{\cdot}432$, 12 d.f.). It was not reasonable to test simultaneously for the joint effects of the mating system and the biogeographic origin because no temperate species is a selfer and the dataset was restricted.

4. Discussion

(i) *Selfing and the loss of genetic variability*

This study shows that the genetic variability as estimated by the number of alleles per population and the gene diversity is reduced in selfing animal populations when compared to outcrossing populations. Selander & Ochman (1983) and Brown & Richardson (1988) reviewing the literature on snails showed that the heterozygosity is lower in selfers than in outcrossers. However, it is not clear whether they were referring to observed heterozygosity or gene diversity, a consideration of importance in selfers. It is noteworthy to mention that the present dataset includes more studies than these two previous references, and especially that some of the studies used by these authors were dropped out when they did not conform to the conditions mentioned in the Materials and Methods chapter (heterozygosity available only, few individuals) which gives more confidence in the results. This extends to animals trends that were uncovered in plants (Hamrick & Godt, 1989; Schoen & Brown, 1991). The tendency towards lower polymorphism in selfers is also indicated by a lower percentage of polymorphic loci. A note of caution is required here as some studies are clearly restricted to polymorphic loci (e.g. Selander & Hudson, 1976). The observed heterozygosity is also lower in selfers as expected. However, as the mating system has been inferred in some studies from genotypic data, observed heterozygosity cannot be considered as independent evidence of a lowered variability. I also found no significant difference between selfers and outcrossers for the coefficient of variation of N_e estimated using the procedure of Chakraborty & Neel (1989), in contrast to the results of Schoen & Brown (1991). The cause may be that the selfing species studied here were actually poorly polymorphic.

Overall a comparison of results on plants and animals indicates similar trends. However, it seems that the loss of variability is even stronger in animals than in plants. For instance gene diversity is divided at least by a factor four (more than ten in freshwater snails) indicating that processes beyond the halving of effective size only are at work (for comparison the general result in plants is a halving of gene diversity). It may be objected that I did not account for the various levels of variation (sampling of genes across loci and populations; see Weir, 1989) that may have obscured the observed result. However, the tremen-

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Table 6. *Number of alleles after a bottleneck. n_1 and n_2 are the number of alleles expected after a bottleneck respectively in outcrossing and selfing populations given Θ, the number of alleles during the bottleneck N and the number of alleles prior to the bottleneck $E(k_1)$ and $E(k_2)$*

	N	n_1	n_2	$n_1/E(k_1)$	$n_2/E(k_2)$
$\Theta = 0{\cdot}16$					
$E(k_1) = 3{\cdot}355$					
$E(k_2) = 2{\cdot}132$	2	1·262	1·138	0·376	0·534
	10	1·531	1·274	0·456	0·597
	100	1·902	1·459	0·567	0·684
	1000	2·271	1·644	0·677	0·771
	10000	2·639	1·829	0·787	0·858
$\Theta = 0{\cdot}01$					
$E(k_1) = 1{\cdot}129$					
$E(k_2) = 1{\cdot}061$	2	1·018	1·009	0·901	0·950
	10	1·035	1·018	0·917	0·959
	100	1·058	1·029	0·937	0·970
	1000	1·082	1·041	0·958	0·981
	10000	1·105	1·052	0·978	0·991

dous loss of variability indicates that this is irrelevant. Striking examples of low variability are the extreme values observed in some species as *Rumina decollata*, *Rivulus marmoratus* and *Bulinus truncatus*. Genetic hypotheses that could explain this discrepancy are genetic hitchhiking and background selection (see below).

(ii) *Bottlenecks in freshwater snails*

The habitats occupied by freshwater snails are patchily distributed. Discontinuity on suitable habitats even in large lakes or rivers is imposed on populations by environmental factors such as water currents, wave action and food availability (Brown, 1994), increasing the patchiness and decreasing the potential size of populations. This is especially true in the tropics where populations experience fluctuations in size and dramatic bottlenecks may be common following drought (Brown, 1994). The halving of gene diversity expected under selfing as a consequence of halving the effective population size has been used above as a null hypothesis. A similar simple hypothesis for bottlenecks cannot be built as their consequences depend on the number of individuals before and after the crash and the growth model of the population (see Nei *et al.* 1975). Obviously the loss of variability, especially gene diversity, is higher for extreme bottlenecks followed by slow growth. However, bottlenecks may be the main reason explaining the very restricted variability in tropical freshwater snails. This could be tested by following allozyme polymorphism in populations submitted to various water regime over a number of generations. Genetic drift following bottlenecks could be detected using standard tests (Waples, 1989). A restriction is the very limited variability available; this may partly explain why the tests failed in *Bulinus globosus* (Njiokou *et al.* 1994).

A similar comparison was not possible in the other groups studied. Though the distribution of land snail populations spans over a gradient of patchiness (*Helix aspersa* is rather continuously distributed whereas *Sphincterochila* spp. are very patchily distributed; Guiller, 1984; Nevo *et al.* 1983) species cannot be contrasted as strongly as in freshwater snails. The limited dataset prevents any serious analysis in the non-Pulmonates species though some Platyhelminth species quite likely are submitted to bottlenecks due to their parasitic mode of life.

(iii) *Selfing and bottlenecks*

To understand the relative role of genetic hitchhiking, bottlenecks and background selection, we can distinguish first between the conditions under which they operate and their genetic consequences. Genetic hitchhiking plays a role only if some initial disequilibrium occurs between the marker loci (here allozymes) and the selected loci. A restriction of genetic polymorphism estimated by the final frequencies at the marker loci holds apparently only at very high selfing rates (Hedrick, 1980). However the role of hitchhiking has to be worked out more completely. Background selection assumes a high enough mutation rate for deleterious recessives at fitness loci in agreement with the few data available obtained from *Drosophila* (Crow, 1993), and applies when the selfing rate is about higher than 75%. Bottlenecks as a process external to the genome have no specific genetic requirements besides a small effective population size. The range of population size over which the three processes mentioned above have a significant role may not overlap. Bottlenecks are important when the size of the population during the bottleneck is small and the population size recovers slowly (see Nei *et al.* 1975). On the other hand background selection can be applied to moderate size populations. Its role and that of hitchhiking in small populations remains unclear though probably obscured by stochastic effects. A critical point is whether there is some threshhold value of the effective size at which both background selection and bottlenecks have a role.

The question is then whether the three processes may be distinguished through their genetic consequences. No test is currently available. As mentioned above, a null hypothesis can be constructed based on the decrease of the effective size as a halving of gene diversity is expected under pure selfing. However no simple prediction can be made on the number of segregating alleles, neither can we infer the

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respective role of different forces when the variability is extremely low. Qualitative predictions have been reviewed by B. Charlesworth *et al.* (1993). Some insight may be gained from the regions of the genome – we are here concerned solely with the nuclear genome – which are affected by the loss of polymorphism and the pattern of variability among populations. If only one locus or one population is concerned with a loss of polymorphism, hitchhiking or a bottleneck may be implicated whereas all selfing populations are affected by hitchhiking and background selection. However, such simple situations are probably more the exception than the rule. It is also generally unknown how far from equilibrium or from the most recent bottleneck a given population is. It is therefore difficult to test for the slight excess of rare alleles expected in the long term for both bottlenecks and hitchhiking when populations are recovering from their allele loss, though probably some bottlenecks may give large rather than slight effect (D. Charlesworth, pers. comm.). No unequivocal solution can therefore be provided from geographical surveys of allozyme variability. Clearly the three effects can be disentangled empirically only when the history of the populations studied is known (this would be true under laboratory conditions).

A further point is that selfing and bottlenecks interact, though no mathematical analysis of the influence of bottlenecks in non-random-mating populations has been developed. The question is whether bottlenecks cause a stronger loss of genetic variability in inbred populations. A simple analysis can be performed as follows for estimating the number of alleles before and after a bottleneck in selfers *v.* outcrossers (see Nei *et al.* 1975). The probability of drawing k allelic types in a sample of z genes knowing N_e and u ($\Theta = 4N_e u$) is given by Ewens's sampling formulae for a random-mating population at mutation/drift equilibrium (Ewens, 1972). A similar probability for purely selfing populations is obtained using $N_e/2$ instead of N_e. Given Θ and sizes before and after the bottleneck, values for selfers and outcrossers can be compared (Table 6). The relative loss of alleles is also given. The absolute values of the number of alleles after bottlenecks is of course lower in selfing populations. This is mainly due to their initial number of alleles being lower as a consequence of their reduced size. However the relative loss of alleles is stronger in outcrossing populations (Table 6). Both the absolute and relative loss of alleles are less marked with decreasing Θ and size before the bottleneck. A more elaborate analysis is of course required to know the role of intermediate selfing rates and the recovery of variability after the bottleneck for both the number of alleles and gene diversity.

(iv) *Selection in small populations*

The role of hitchhiking and background selection makes clear that selection at some loci influences the amount of neutral variability maintained. In populations of very small size these two genetic processes are overwhelmed by stochastic processes. Mutation accumulation resulting from the fixation of deleterious alleles at loci with effects on fitness may indeed occur through genetic drift. Muller's ratchet may also operate in small populations when recombination is limited. Both processes lead to an increase of the genetic load and may decrease the size of populations under any kind of hard selection. This runaway mechanism has been called mutational meltdown when Muller's ratchet is concerned (Gabriel *et al.* 1993) and may be another cause of decreased variability through a decrease in size that accelerates the role of bottlenecks. A basic assumption is of course that selfers accumulate deleterious mutations at a higher rate than outcrossers. This seems to be true for mutation fixation though not for Muller's ratchet when the population size is very small (of the order of 100). However, the detailed analysis of D. Charlesworth *et al.* (1993) indicates that both Muller's ratchet and mutation fixation are expected to occur only under restricted conditions on the selfing rate, mutation rate and population size, and therefore the conditions under which neutral variability could be lost. It remains to be shown whether these conditions correspond to any realistic situations for natural populations. For instance small populations may disappear for demographic reasons leaving few chances for population geneticists to analyse the role of mutation accumulation on neutral variability.

(v) *The maintenance of genetic variability above the population level*

A classical result mentioned in the Introduction is that selfing redistributes population genetic variation into separate inbred lines. This has been extended to the population level, that is selfers show higher values of population structure indices than outcrossers. This tendency has been empirically substantiated by the survey of Hamrick & Godt (1989): G_{ST} values were higher in selfers than in any other species (including outcrossers). The results here are similar though the difference is not always significant. However Maruyama & Tachida (1992) have shown under the island model with no extinction and assuming that mutation (u) is smaller than migration (m) that:

$$G_{ST} = \frac{1}{1+4Nm(1-S/2)},$$

where S is the selfing rate and N the effective

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population size. This formula is equivalent at equilibrium under the mixed mating model to:

$$G_{ST} = \frac{1}{1+4(N/1+F)m}.$$

G_{ST} is expected to be doubled in selfers compared to outcrossers when m is large. No difference is expected when migration is limited. Therefore the increase of G_{ST} observed here and part of that observed by Hamrick & Godt (1989) may simply be a consequence of the increase of the effective size. However, that the genetic variability is distributed in a different way does not imply that some of the variability lost at the population level may be stored at a higher level (group of populations). By comparison to what is known at the population level, the relative variability stored in a metapopulation may be estimated through its effective size in selfers and outcrossers. This can be done from equations (27) and (28) of Maruyama & Kimura (1980) equating the effective size of a haploid group of populations with that of a purely selfing population. The model assumed is the infinite-allele model. With no extinction, the ratio of the effective size of a selfing metapopulation over an outcrossing one is:

$$R = \frac{(n+1)N_c+(n/u+m)}{2(n+1)N_c+[n/2(u+m)]},$$

where N_c is the effective size of each outcrossing subpopulation (I assumed $N_s = N_c/2$ with N_s the effective size of each selfing subpopulation), m is the migration rate among subpopulations and n is the number of subpopulations in both the selfing and outcrossing metapopulation. It can easily be shown that $R = 2$ when $N_c \ll 1/m$, whereas $R = 1/2$ when $N_c \gg 1/m$ and negligible mutation. This result suggests that, as long as the mean effective size of subpopulations and the migration rate are unknown, no prediction can be made on the amount of variability that can be maintained at the level of a group of populations. I assumed here that selfers and outcrossers have identical migration rate which may be true in snails but not in many plants in which pollen flow may be far higher in outcrossers than in selfers (see e.g. Govindaraju, 1988). Moreover when gene flow results from the movement of diploid propagules, the actual gene flow is divided in selfing populations by a factor $1+F$ when compared to outcrossing populations, because of the correlation among alleles at a given gene when inbreeding. This even increases the possible variations of R and of the ratio of G_{ST} in selfing over outcrossing populations. The variability stored at the metapopulation level can be analysed by comparing the number of alleles per population and the number of alleles per study. The ratio of these values estimated per polymorphic loci does not significantly differ between selfers and outcrossers among both freshwater and land snails showing that polymorphism is lost at the two levels alluded to above.

(vi) *Other markers*

Data on genetic polymorphism relevant to our study have been obtained with allozymes. Besides the possibility that these may be under selective pressure, their mutation rate is so low that the polymorphism in highly selfing populations is very limited, often precluding tests for the parameters involved in the loss of polymorphism. More polymorphic markers would therefore be useful. Few attempts have been made to correlate DNA polymorphisms and mating systems. On a very limited dataset Ritland, Ritland & Straus (1993) found no correlation using rDNA sequences. Promising candidates for our purpose are microsatellites provided that under their peculiar mode of mutation and high rate of mutation the expectations from the models cited above still hold. Taylor, Sherwin & Wayne (1994) recently showed that the level of variation in a bottlenecked mammal species (*Lasiorhinus kreftii*) at sixteen loci is very low compared to a closely related species. However, both gene diversity and the number of alleles remains far higher than the mean values observed in mammals when using allozyme electrophoresis (Nevo *et al.* 1984). Turner *et al.* (1992) characterized the molecular variation of *Rivulus marmoratus* using multilocus microsatellite and minisatellite probing. They showed some variation, whereas zero variation was found by Vrijenhoek (1985) in the same species at more than thirty allozyme loci (Appendix 3). Single-locus microsatellite analysis in another highly selfing species, the freshwater snail *Bulinus truncatus*, indicates similar tendency, that is a large number of alleles per locus and some observed heterozygosity (F. Viard & P. Jarne, unpublished results) whereas Njiokou *et al.* (1994) found little variation (Appendix 1). Clearly the mutation rate of microsatellites is high enough to prevent exhaustion of the variability even at high selfing rates and in populations regularly submitted to crashes. They will therefore be very useful in studies to understand how variability is lost from small inbred populations.

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Appendix 1. *Genetic variability and mating systems in hermaphroditic freshwater snail species*

Species	$N_{pop.}$	$N_{loc.}$ (pl)	$N_{all.}$	H_o	H_e	G_{ST}	Mating system	Authors
*Biomphalaria alexandrina**	11	28 (21)	1·15 (0·04)	0·051 (0·021)	0·056 (0·022)	0·127 (0·113)	C	Vrijenhoek & Graven (1992)
Biomphalaria camerunensis	12	19 (26)	1·06 (0·05)	0·008 (0·012)	0·016 (0·021)	0·761 (0·044)	C	Mimpfoundi & Greer (1990*c*)
*Biomphalaria glabrata**	7	26 (15)	1·13 (0·33)	< 0·055 (—)	0·021 (0·022)	0·470 (0·355)	C	Mulvey & Vrijenhoek (1982)
*Biomphalaria glabrata**	6	21 (57)	1·20 (0·09)	0·017 (0·013)	0·020 (0·015)	0·463 (0·419)	C	Mulvey *et al.* (1988)
Biomphalaria pfeifferi	12	12 (100)	1·28 (0·15)	0·007 (0·008)	0·032 (0·030)	0·279 (0·221)	S	Bandoni *et al.* (1990)
*Biomphalaria pfeifferi**	19	19 (21)	1·02 (0·03)	0·001 (0·005)	0·006 (0·013)	·0866 (0·190)	S	Mimpfoundi & Greer (1990*b*)
*Biomphalaria straminea**	4	19 (74)	1·35 (0·08)	0·082 (0·018)	0·081 (0·017)	0·082 (0·055)	C	Woodruff *et al.* (1985)
*Bulinus cernicus**	25	6 (83)	1·37 (0·43)	0·090 (—)	0·097 (0·091)	0·321 (0·239)	C	Rollinson & Wright (1984)
*Bulinus cernicus**	8	11 (64)	1·38 (0·54)	0·009 to 0·203	0·085 (0·068)	0·320 (0·252)	C	Rollinson *et al.* (1990)
Bulinus forskalii	10	15 (0)	1·00 (0·00)	0·000 (0·000)	0·000 (0·000)	—	S	Mimpfoundi & Greer (1989)
Bulinus forskalii	32	15 (20)	1·02 (0·05)	0·001 (0·004)	0·008 (0·016)	0·751 (0·217)	S	Mimpfoundi & Greer (1990*a*)
*Bulinus globosus**	15	6 (50)	1·12 (0·16)	0·036 (0·056)	0·039 (0·053)	0·315 (0·323)	C	Jelnes (1986)
*Bulinus globosus**	13	18 (50)	1·52 (0·95)	0·034 (0·019)	0·029 (0·026)	0·547 (0·323)	C	Njiokou *et al.* (1994)
Bulinus senegalensis	7	15 (0)	1·00 (0·00)	0·000 (0·000)	0·000 (0·000)	—	S	Mimpfoundi & Greer (1989)
Bulinus truncatus	9	14 (21)	1·07 (0·27)	0·000 (0·000)	< 0·001 (—)	0·007 (0·000)	S	Jelnes (1986)
Bulinus truncatus	13	24 (8)	1·00 (0·00)	0·000 (0·000)	0·000 (0·000)	1·000 (0·000)	S	Mimpfoundi & Greer (1990*d*)
*Bulinus truncatus**	18	42 (21)	1·01 (0·03)	0·000 (0·000)	0·005 (0·010)	0·863 (0·306)	S	Njiokou *et al.* (1993)

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Appendix 1. (*Cont.*)

Species	$N_{pop.}$	$N_{loc.}$ (pl)	$N_{all.}$	H_o	H_e	G_{ST}	Mating system	Authors
*Bulinus umbilacatus**	6	7 (43)	1·23 (0·36)	0·071 (0·049)	0·079 (0·037)	0·279 (0·165)	C	Jelnes (1986)
Helisoma anceps	1	26 (23)	1·27 (0·53)	0·051 (—)	0·057 (—)	—	C	Mulvey *et al.* (1987)
Lymnaea auricularia	4	11 (36)	1·11 (0·19)	0·037 (0·029)	0·075 (0·057)	0·170 (0·147)	M	Coutellec-Vreto *et al.* (1994)
Lymnaea elodes	3	15 (40)	1·49 (0·04)	0·090 (0·01)	0·113 (0·015)	0·200 (0·191)	C	Brown & Richardson (1988)
*Lymnaea peregra**	11	11 (100)	2·00 (0·32)	0·206 (0·200)	0·243 (0·092)	0·183 (0·104)	C	Coutellec-Vreto *et al.* (1994)
*Lymnaea peregra**	4	12 (75)	2·17 (0·12)	0·208 (—)	0·233 (0·020)	0·019 (0·027)	C	Jarne & Delay (1990)
*Physa heterostropha**	10	10 (100)	2·01 (2·33)	0·210 (0·047)	0·260 (0·068)	0·276 (0·120)	C	Dillon & Wethington (unpubl. manuscript)

The parameters describing the genetic variability are defined in Table 1 and text. *pl* refers to the percentage of polymorphic loci. C, M and S means cross-fertilization, mixed-mating and self-fertilization respectively.
* Denotes studies that have been used to estimate the variation of the effective population size (see text) and – denotes missing data.

Appendix 2. *Genetic variability and mating systems in hermaphroditic landsnail species. Legend as in Table 1 and Appendix 1.*

Species	$N_{pop.}$	$N_{loc.}$ (pl)	$N_{all.}$	H_o	H_e	G_{ST}	Mating system	Authors
*Arianta arbustorum**	16	7 (1·0)	2·15 (0·12)	—	0·281 (0·056)	0·163 (0·230)	C	Arter (1990)
*Arion ater**	33	13 (0·31)	1·61 (0·52)	0·058 (0·060)	0·064 (—)	0·405 (0·102)	M	Foltz *et al.* (1982)
Arion circumscriptus	4	18 (0·0)	1·00 (0·0)	0·0 (0·0)	0·0 (0·0)	—	S	McCracken & Selander (1980)
Arion intermedius	5	20 (0·0)	1·00 (0·00)	0·000 (0·000)	0·000 (0·000)	—	S	McCracken & Selander (1980)
Arion subfuscus A	5	13 (0·21)	2·00 (0·58)	0·070 (0·041)	0·077 (—)	0·373 (0·078)	C	Foltz *et al.* (1982)
*Bradybaena fruticum**	11	13 (0·54)	2·08 (0·34)	0·143 (0·042)	0·140 (0·043)	0·324 (0·234)	C	Falniowski *et al.* (1993)
*Cepaea nemoralis**	29	9 (0·33)	1·23 (0·12)	—	0·074 (0·036)	0·357 (0·189)	C	Brussard (1975)
*Cepaea nemoralis**	25	9 (1·00)	1·96 (0·31)	≈ 0·176 (—)	0·182 (0·096)	0·418 (0·235)	C	Guiller & Madec (1993)
Cerion bendalli	3	22 (0·23)	1·17 (0·05)	0·055 (0·008)	0·057 (0·015)	0·082 (0·082)	C	Woodruff (1975)
Chondrina clienta	6	17 (0·18)	1·00 (0·00)	0·000 (0·000)	0·000 (0·000)	1·000 (0·000)	S	Baur & Klemm (1989)
Deroceras caruanae	2	13 (0·23)	1·11 (0·05)	0·040 (0·000)	—	0·023 (0·013)	C	Foltz *et al.* (1984)
Deroceras reticulatum	17	11 (0·73)	2·21 (0·22)	0·196 (0·039)	0·197 (—)	0·163 (0·145)	C	Foltz *et al.* (1984)
Helix aspersa	8	5 (1·0)	2·75 (0·38)	—	0·411 (0·078)	0·185 (0·086)	C	Selander & Kaufman (1975)
*Helix aspersa**	58	14 (0·79)	1·9 (0·29)	0·152 (0·039)	0·183 (0·039)	0·312 (0·150)	C	Guiller (1994)
Heterostoma paupercula	16	3 (1·00)	2·8 (1·11)	—	0·313 (0·138)	0·482 (0·172)	M	Cook & Lace (1993)
Liguus fasciatus	7	24 (0·04)	1·07 (0·02)	0·005 (0·006)	0·013 (0·012)	0·455 (0·000)	M	Hillis *et al.* (1987)
Mandarina aureola	34	21 (0·57)	1·24 (0·16)	0·051 (0·033)	0·061 (0·040)	0·228 (0·225)	C	Chiba (1994)
Mandarina ponderosa	11	21 (0·38)	1·26 (0·07)	0·070 (0·017)	0·075 (0·020)	0·111 (0·159)	C	Chiba (1994)

Appendix 2. (*Cont.*)

Species	$N_{pop.}$	$N_{loc.}$ (pl)	$N_{all.}$	H_o	H_e	G_{ST}	Mating system	Authors
Milax budapestensis	2	8 (0·63)	1·46 (0·11)	0·12 (0·07)	0·130 (—)	0·175 (0·131)	C	Foltz *et al.* (1984)
Rumina decollata	18	25 (0·40)	1·00 (0·01)	< 0·001 (< 0·001)	< 0·001 (< 0·001)	0·996 (0·011)	S	Selander & Kaufman (1973)
*Rumina decollata**	20	5 (1·00)	1·95 (0·22)	0·049 (0·047)	0·198 (0·158)	0·329 (0·048)	S	Selander & Hudson (1976)
Samoana jackieburchi	1	20 (0·65)	1·5 (0·00)	0·135 (—)	0·108 (0·193)	—	C	Johnson *et al.* (1986)
Sphincterochila aharonii	2	28 (0·46)	1·21 (0·10)	0·042 (0·035)	0·057 (0·021)	0·282 (0·313)	C	Nevo *et al.* (1983)
Sphincterochila cariosa	2	28 (0·36)	1·29 (0·05)	0·054 (0·021)	0·065 (0·010)	0·260 (0·263)	C	Nevo *et al.* (1983)
Sphincterochila fimbriata	3	23 (0·74)	1·68 (0·40)	0·104 (0·038)	0·139 (0·071)	0·383 (0·279)	C	Nevo *et al.* (1983)
Sphincterochila prophetarum	3	28 (0·86)	1·81 (0·31)	0·088 (0·039)	0·109 (0·063)	0·289 (0·296)	C	Nevo *et al.* (1983)
Sphincterochila zonata	2	28 (0·50)	1·52 (0·03)	0·077 (0·041)	0·099 (0·052)	0·153 (0·203)	C	Nevo *et al.* (1983)
Theba pisana (Australia)	8	25 (0·16)	1·14 (0·02)	—	0·047 (0·011)	0·221 (0·054)	C	Johnson (1988)
*Theba pisana** (worldwide)	8	25 (0·68)	1·49 (0·29)	0·101 (0·053)	0·103 (—)	0·241 (0·194)	C	Johnson (1988)
Triodopsis albolabris	6	8 (0·50)	1·39 (0·12)	—	0·097 (0·070)	0·132 (0·129)	C	McCracken & Brussard (1980)
Xerocrassa seetzenii	3	20 (0·60)	1·67 (0·19)	0·078 (0·015)	0·117 (0·087)	0·211 (0·185)	C	Nevo *et al.* (1981)

≈ means that H_o or H_e has been inferred from F_{IS} (see text).

Appendix 3. *Genetic variability and mating systems in hermaphroditic animal species* (*Pulmonata are excluded*)

Phylum Species	$N_{pop.}$	$N_{loc.}$ (pl)	$N_{all.}$	H_o	H_e	G_{ST}	Mating system	Authors
Polychaetes								
Pileolaria pseudomilitaris	21	7 (0·29)	2·8 (0·24)	—	0·292 (0·082)	0·141 (0·076)	C	Beckwitt (1980)
Hediste (*Nereis*) *diversicolor*	2	10 (0·30)	1·40 (0·14)	0·111 (0·125)	0·097 (0·105)	0·393 (0·324)	C	Fong & Garthwaite (1994)
Hediste (*Nereis*) *limnicola*	4	9 (0·33)	1·30 (0·14)	0·050 (0·046)	0·060 (0·049)	0·197 (0·161)	M	Fong & Garthwaite (1994)
Helminths								
Mesostoma ehrenbergii	2	11 (0·18)	1·05 (0·06)	0·008 (0·011)	0·022 (0·032)	0·299 (0)	S	Hebert & Benton (1990)
Mesostoma lingua	32	10 (0·20)	1·2 (0·02)	0·072 (0·023)	0·069 (0·022)	0·175 (0·058)	C	Hebert & Payne (1985)
Fascioloides magna	14	5 (1·00)	2·4 (0·07)	≈ 0·35 (—)	0·361 (0·034)	0·027 (0·023)	C	Mulvey *et al.* (1991)
Paragonimus ohirai	4	18 (0·39)	1·26 (0·21)	0·058 (0·028)	0·053 (0·021)	0·098 (0·062)	C	Agatsuma & Habe (1986)
Bivalves								
Ellipsio complanata	16	14 (0·57)	1·9 (0·20)	—	0·170 (0·046)	0·143 (0·078)	C	Kat & Davis (1984)
Musculum partumeium	2	22 (0·23)	1·16 (0·22)	0·040 (0·056)	≈ 0·03 (—)	0·246 (0·146)	S	McLeod *et al.* (1980)
Vertebrata-fishes								
Campostoma anomalum	7	17 (0·29)	1·33 (0·03)	—	0·089 (0·036)	0·261 (0·191)	C	Zimmerman *et al.* (1980)
Campostoma oligolepis	5	17 (0·24)	1·24 (0·0)	—	0·079 (0·008)	0·202 (0·146)	C	Zimmerman *et al.* (1980)

Appendix 3. (*Cont.*)

Phylum Species	$N_{pop.}$	$N_{loc.}$ (pl)	$N_{all.}$	H_o	H_e	G_{ST}	Mating system	Authors
Chrysichthys maurus	12	19 (0·58)	1·24 (0·17)	—	0·035 (0·032)	0·324 (0·430)	C	Agnese (1989)
Poeciliopsis monacha	8	25 (0·44)	1·17 (0·05)	—	0·045 (0·027)	0·337 (0·194)	C	Vrijenhoek (1979)
Rivulus marmoratus	12	31 (0·13)	1·00 (0·00)	0·000 (0·000)	0·000 (0·000)	1·000 (0·000)	S	Vrijenhoek (1985)

Legend is as in Table 1 and Appendix 1.

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