Ease of fixation of a change in coiling: computer experiments on chirality in snails

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This paper deals with chirality of snails. It explores the ease of fixation of a change in coiling direction, caused by an invasion of snails carrying a mutant chirality allele into a normal, homogeneous population, by using Monte-Carlo simulation. Additionally, single-gene speciation on the basis of chirality is discussed. Six factors are studied in particular, namely: (i) the absolute and relative population size; (ii) the mating success, which is known to be related to shell shape, especially the height/width ratio; (iii) the maternal effect, underlying chirality; (iv) the (low) mobility of the snails; (v) fitness differences (heterosis); and (vi) the invading mutant allele being either dominant or recessive. The impact of these factors is quantified. Small populations with not too few invaders and dominance of the mutant chirality allele are of paramount relevance for its occasional fixation. In comparison to this, the maternal effect turns out to be considerably less significant, whereas the mobility of the snails plays only a minor role. The simulations confirm the expectation that heterosis can be very effective. Logically, the mating success is also a very important factor. This reflects the observation in nature that among snails with slender shells, when mating between mirror-image individuals is still possible to a certain extent, reverse populations have originated far more frequently than among snails with globular shells, where such matings are impossible. Only the latter cases, which are very rare in nature, may concern single-gene speciation.

Keywords: chirality, maternal effect, reproductive isolation, snails, speciation.

Introduction

Single-gene mutations resulting in complete reproductive isolation between members of successive generations, without being inferior or lethal, are rare. A relatively well-known example concerns the gene that is responsible for chirality, the direction of coiling, in snails. In the few cases that have been studied genetically, it turns out that chirality is determined by a pair of alleles at a single gene-locus. Both right or clockwise (dextral) and left or counterclockwise (sinistral) coiling may be dominant. Dextral is by far the more common form in nature: when the shell is kept upright, its aperture is situated below at the right side. Robertson (1993) discussed the different bauplans which may be hidden behind the two shell phenotypes. From his data we may conclude that far more than 90 per cent of the taxa are normally dextral; more exact

percentages are not known. With regard to chirality, a maternal effect was shown to occur, that is, the genotype of the egg-producing parent controls the direction of coiling of the offspring. Thus for example, an individual that is genotypically homozygous for sinistral, will be phenotypically dextral if its female parent was heterozygous with respect to chirality and dextral is dominant. See Asami (1993) for further details.

In snails with globular shells, such as *Helix pomatia* (Fig. 7) and *Arianta arbustorum*, dextral and sinistral individuals may show total premating isolation (Gittenberger, 1988). This is caused by the shape of the shells and the resulting positions of the animals physically preventing copulation. Strictly adhering to the most commonly accepted species concept, mirror-image specimens cannot be considered conspecific anymore in such cases, when (indirect) gene flow between the two forms has stopped completely. This situation is reached when heterozygotes no longer occur in the (sub)popula-

tions of the two forms and the shape differences prohibit successful copulation. From that moment on the two entities will evolve as separate lineages, although initially their genomes will still be very similar.

In snails with slender, spindle-shaped shells (Fig. 8), mating between mirror-image specimens is still possible, but may be less successful (Johnson, 1982; Asami, 1993). This implies that in these cases gene flow even between two homozygous populations of opposite coil will occur when there is (secondary) contact. Such populations are considered conspecific, as for example in *Partula suturalis* (see Johnson *et al.*, 1990).

Gittenberger (1988) listed a series of observations concerning chirality, suggesting that populations with a single or very few mirror-image specimens, entire populations of opposite coil, and sister species of opposite coil, might exemplify stages in a process of speciation on the basis of chirality. Additionally he emphasized that the obvious minority of taxa with sinistral shells is not randomly distributed among the gastropods, but concentrated in groups that have slender, spindle-shaped shells. This observation was neglected by Johnson *et al.* (1990) and Orr (1991), while discussing the relationship between speciation and chirality.

On the basis of simulations, Johnson *et al.* (1990, p. 464) concluded that chirality 'is particularly unlikely to serve as the basis for sympatric speciation'. Also using simulations, Orr (1991) argued that 'single-gene speciation', based on gastropod chirality and maternal inheritance, is possible. He did so for the cases in which opposite coil implies a complete premating isolation and allowed 5 per cent of all pairings to involve selfing. Asami (1993, p. 265), assuming that the dextral allele is usually dominant, suggested that 'divergence of left-handed groups may have been associated with evolution of dominant sinistrality'.

The present paper aims to reconsider the conclusions of Johnson *et al.* (1990) and Orr (1991), which are not entirely congruent, and obtain a better insight with regard to some factors that may be relevant to the ease of fixation of a mutant chirality allele by providing additional, quantified data. A simulation model allowed us to study the effects of: (i) differences in both the absolute and the relative number of mutant individuals introduced into populations of various sizes; (ii) the mating success in opposite coil encounters (not a simple yes or no, but varying from 0 to 100 per cent and thought to be related to shell shape); (iii) the maternal effect (present or not); (iv) the mobility of the individuals

within the population (related to inbreeding); (v) fitness of the different genotypes; and (vi) the genetic background of chirality, i.e. the mutant allele being dominant or recessive. The invaders in our simulation experiments represent the initial carriers of a mutant allele. The first mutant individuals will be heterozygous and, therefore, the invaders are supposed to be so. Discussing premeiotic clusters of mutation, with the implication that sometimes a single individual produces a cluster of mutant gametes, Woodruff & Thompson (1992) gave support to our assumption that occasionally groups of mutants ('invaders'), descendants of a single clutch, may enter the population. The distributional patchiness of many species of terrestrial snails (Jones *et al.*, 1977; Schilthuizen & Lombaerts, 1994) gives support to the assumption of populations evolving in considerable isolation. We will deal with clusters of mutant invaders from outside and mutants originating within the population.

Although self-fertilization is known to occur in hermaphroditic snails and could easily be implemented in the model, we did not do so. It would not be realistic with regard to the majority of the pulmonate species to accept simply a certain percentage of selfing. In laboratory experiments, selfing turns out to be possible in several species, after a relatively long period of isolation of the adult individual; it may be accompanied then by a considerable fitness reduction (Tompa, 1984; Chen, 1994). Nevertheless, selfing may be relevant when a mirror-image individual cannot find a mate. This study is restricted to situations where finding a mate is not problematic.

Materials and methods

A computer program was designed to perform Monte-Carlo simulation of the invasion of mirror-image individuals in a uniform snail population. We used the programming language APL because it is powerful and enables changes to be made very quickly. As APL is an interpreted language, its execution speed cannot compete with that of a compiled language. However, the speed of program development, debugging and repeatedly improving, far outweighted the disadvantage of slower execution.

The program simulated a genotypically and phenotypically homogeneous population of hermaphroditic snails into which a smaller number of different snails invades. Subsequently the program simulated several generation cycles that repeated the following steps.

1 Start an initial population of a certain size N with RR individuals only. Here RR stands for 'genotypically and phenotypically dextral (right-coiled)'.

2 Introduce a (smaller) number of different invaders. We used HR as default. HR stands for 'genotypically hybrid and phenotypically dextral'. Abbreviations like 15RR/5HR will be used to describe an experiment; it means that an area with capacity 15, occupied by 15 snails RR, is invaded by 5 HR snails. Because the area has a capacity of 15, it contains 20 snails only in the first step, whereas in each subsequent generation only 15 snails survive. Thus, a situation is simulated in which, for example, a bird carries some invading snails, or an island population is enriched by specimens from a piece of driftwood washed ashore; the population increases in generation 1, whereas its sustainable size is restored in the next generation. In another scenario a mutant originates within a population. In the latter case the population remains at its standard size all the time. We simulated both alternatives for various parameter settings and found no significant ($\alpha = 0.05$) difference (two-sided sign-test, $n = 100$, $z = 1.7$, $P = 0.0891$). We choose the former scenario in all subsequent simulations.

3 Mix the secondary population thoroughly and form random pairs. For example, a population of initially 25 RR enlarged by 5 HR would give 15 pairs.

4 Remove all unsuccessful pairs, that is, all incompatible combinations -R with -L.

5 Produce the set of all potentially possible genotypes for each of the remaining pairs: this produces four genetical combinations for each remaining pair.

6 Determine the associated phenotypes by taking into account the maternal effect. One set of four snails is assumed to be produced by one parent (their phenotype is determined by the genotype of that particular parent) and another set of four is assumed to get the phenotype of the other parent. So each pair produces eight descendants.

7 Realize the next generation by random choice of N individuals from the potentially possible set of descendants as generated in step 6. Here N is a previously given population size.

8 Repeat this procedure for the next generation, starting from step 3, until the population is homogeneous.

For each parameter setting we computed 100 simulations. A simulation ended after 600 generations or when sinistral or dextral alleles disappeared (whichever occurred first). We recorded the percentage of those 100 runs that ended with a population

of homozygous mutant snails. Each of these 'evolutionary experiments' was repeated 10 times (or occasionally 20 times, if the variance was too high), and the mean of the percentages was computed. We also noted how many generations were required on average before the populations reached uniformity.

Absolute and relative population size

The relevance of the absolute and that of the relative number of invaders was investigated by performing steps 1–8 as indicated (Fig. 1).

Mating success

To study the implications of the observation that snails with globular shells may have no or hardly any success in attempts to mate with a mirror-image individual, whereas snails with slender, spindle-shaped shells may have more success in such a situation, another version of the program was developed, in which we changed step 4 as follows.

4' Remove only a certain percentage of the pairs -R and -L, so that a fraction P_{ms} determined by the probability of mating success remains.

This version gives the possibility of comparing simulations with different mating successes, which reflect populations of snails differing with respect to shell shape (Fig. 2).
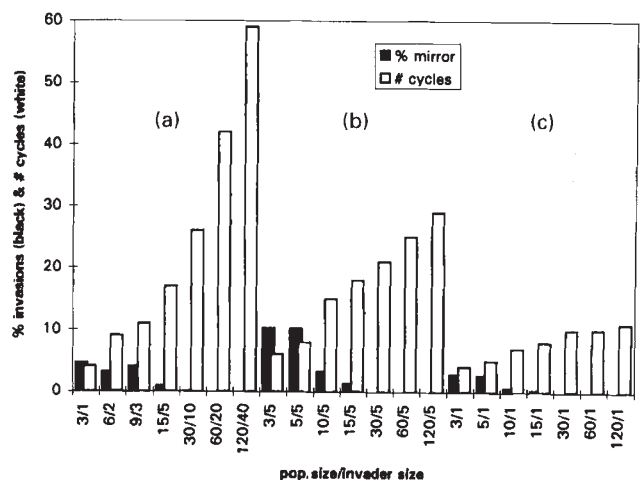

Fig. 1 Percentages of successful invasions by mirror-image snails, for various population/invader numbers. Black bars show the percentages of successful invasions (numbers on the vertical axis are per cent). White bars show the number of generations (cycles) needed for the population to become homogeneous (numbers on the vertical axis are cycles). In (a) population size increases with a constant ratio 3:1; (b) shows increasing population sizes with constantly five invaders; (c) is similar to (b), but has only a single invader each time.

Maternal effect

To study the importance of the maternal effect in achieving mutant allele fixation, we introduced an alternative version of the original program, changing step 6 as follows.

6′ Next determine the associated phenotypes without maternal effect, which implies that the chirality of each descendant is determined by its own genes.

By comparing simulations including step 6′ with those using step 6, the consequence of the maternal effect becomes visible (Fig. 3).

Mobility

In the previous simulations the population was thoroughly mixed in step 4. This might not always be realistic for snails, however. If a population is spread over an area that is relatively large in relation to the individual mobility of the animals, there will be a more restricted mixing of individuals. To test the relevance of mobility, we placed all snails along a line and changed the way of mixing in step 3 as follows.

3′ Mix according to mobility parameter M.

For $M = 1$, each snail can only move one position to each side or not at all, for $M = 5$, each snail can move none to five positions to the left or right. All positions have equal probability of being chosen (uniform distribution).

The introduction of the mobility parameter gives the possibility of studying the effect of more or less important inbreeding, whereas a high value for M simulates a situation with a complete mix (Fig. 4). So for $M = 5$, the maximum range of any individual is 10. For example, if snail 3 moves the maximum of five positions to the right, it can just mate with snail 13, if that one at the same time moves five to the left. An offspring is placed in the position of the parents; before mating there is a more or less restricted mixing, in conformity with the value of M.

No other things were changed. Thus, in the mobility variant, the invaders initially stored at one end of the line, and their descendants, mix more or less slowly depending on the mobility parameter M.

Fitness

The various genotypes and phenotypes might differ in fitness, which will influence the success of the mutant invader allele. It is obvious that a lower
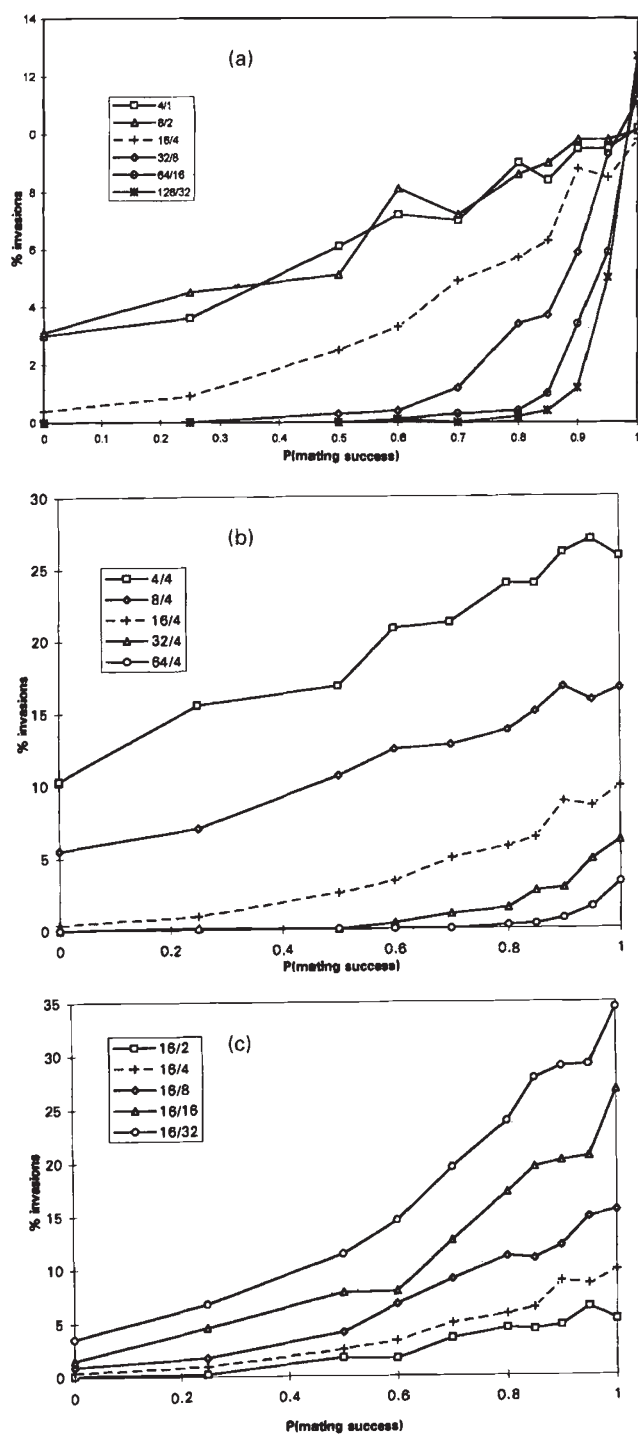

Fig. 2 Percentages of successful invasions by mirror-image snails, depending on mating success. All populations are RR invaded by HR. (a) Population size increases with a constant ratio 4:1; (b) shows increasing population sizes with constantly four invaders; (c) shows an increasing number of invaders with a constant population size. Experiment 16RR/4HR is used as a standard measure and shown with a dotted line in each figure.
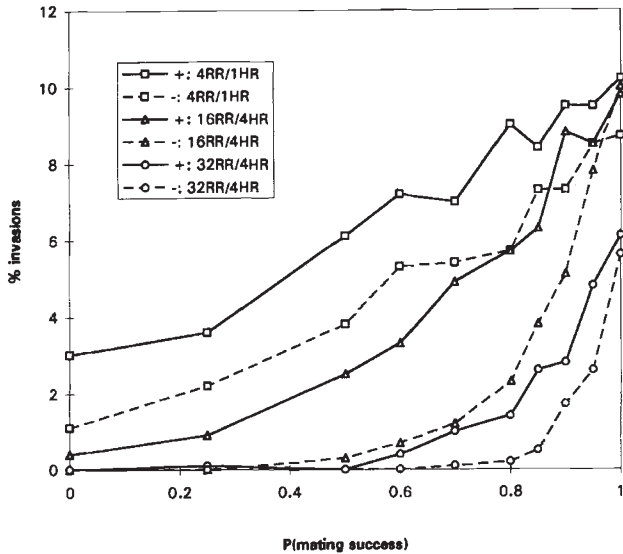
Fig. 3 Percentages of successful invasions by mirror-image snails, with (+, normal line) or without (−, dashed line) maternal effect.

fitness of invaders will hamper fixation, whereas a lower fitness of the original population will improve the chances for the invaders (Fig. 5). Less obvious, however, is the effect of differences in fitness of the hybrids. To study this, we developed a version of the program with step 7 changed as follows.

7′ Realize the next generation by a random choice which (dis)favours hybrids by a certain percentage over homozygous descendants.

Dominance of the chirality allele

Very little is known about the dominance of the alleles for dextral or sinistral coiling. It has been determined, however, that both directions of coiling may be either dominant or recessive genetically (Asami, 1993). To investigate the consequences of an invader carrying a dominant instead of a recessive mutant chirality allele, we carried out an additional simulation study of a uniform population LL that is invaded by a hybrid with a dominant allele (Fig. 6).

Results

Absolute and relative population size

The relevance of absolute and relative population size is clearly shown in Fig. 1. Naturally, the relative frequency of the invader is relevant (Fig. 1b,c). It is
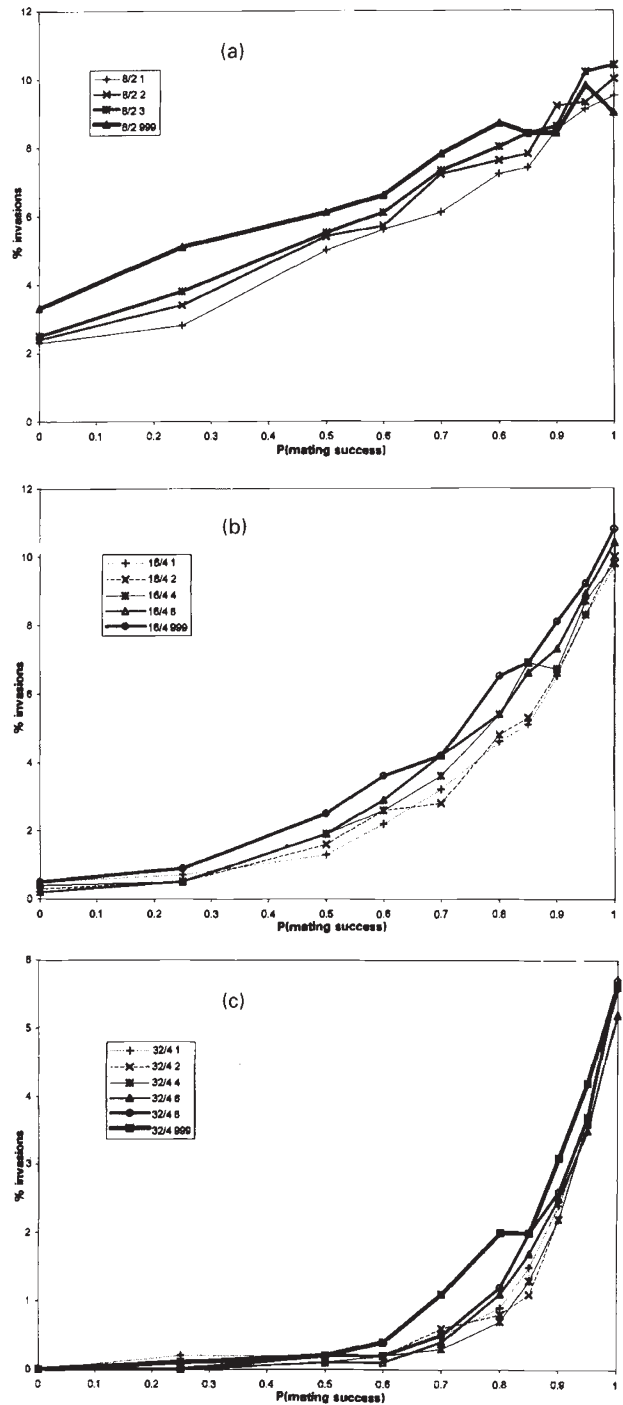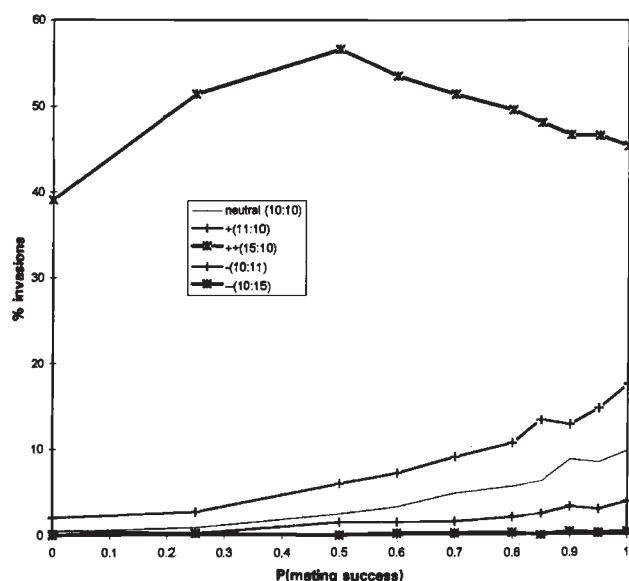

Fig. 4 Percentages of successful invasions by mirror-image snails, depending on mobility. An increasing thickness represents an increasing mobility. M-values range from 1 to 999 (panmictic).

also clear that in areas with a low absolute number of individuals the invading mutant allele has the highest probability of becoming fixed; see for example the simulation experiments 3/1 or 9/3 versus 30/10 (Fig. 1a). The results additionally indicate that
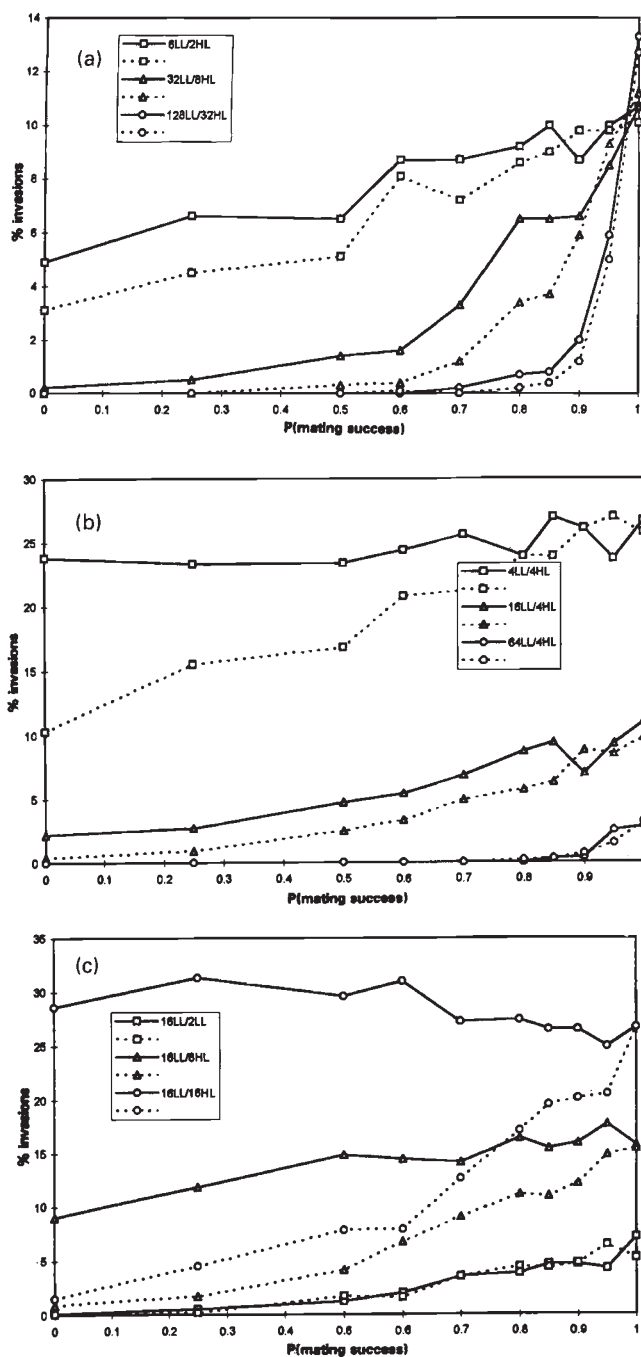
Fig. 5 Percentages of successful invasions by mirror-image snails with a different fitness for hybrids versus homozygotes. Fitness ratios of hybrids versus homozygotes are 15:10 (+ +), 11:10 (+), 10:11 (−) and 10:15 (− −). The results for equal fitness (10:10) are given as a reference. The results of the 16RR/4HR experiments are shown.

within a larger population it takes more time to exterminate the invading allele completely; the required number of generations is much higher in the 120/ than it is in the 3/ (Fig. 1a–c). Even if an allele is brought into a big population by a single invader, like in 120/1, it takes more time to expel it than in small populations like in 3/1 (Fig. 1c).

Mating success

The influence of a varying mating success between mirror-image individuals is shown in Fig. 2. The results show that, except for the smallest populations, roughly speaking, the invasion has a much higher chance of being successful when (clearly) more than one out of each two encounters results in successful mating ($P_{ms} > 0.5$). Of course, the improvement in mutant allele fixation increases with a higher percentage of invaders as compared with the population size (Fig. 2b) and with a higher mating success (Fig. 2a–c). The latter effect becomes far more pronounced in larger populations. As is indicated in for example Fig. 2a, showing simulations with a constant ratio of invaders for various population sizes, fixation of the mutant allele is most probable in large populations when the mating success is very high. A lower mating success gives a drop in fixation percentage in all cases. However,

Fig. 6 Percentages of successful invasions by mirror-image snails, when the invader's coiling direction is dominant (normal lines) or recessive (dotted lines). (a) Population size increases with a constant ratio 4:1; (b) shows increasing population sizes with constantly four invaders; (c) shows an increasing number of invaders with a constant population size.

this is more dramatically so in the largest populations, whereas in (very) small populations the decrease is (far) more gradual (compare $P_{ms} = 0.5$ and $P_{ms} = 1.0$ in Fig. 2a).

Maternal effect

When the results of simulation experiments both with and without maternal effect are compared, it turns out that the chances for a successful introduction of the mutant allele do increase because of the maternal effect. This is most clearly so in cases with a moderate mating success, in small populations, and disappears when the mating success approaches its maximum. Fig. 3 gives the results of a representative subset of the various simulations. The figure shows that the effect is moderate compared with the influence of different values for P_{ms}.

Mobility

On the whole, the degree of mobility did not play an important role in the success of invasions. Although we found several occasions where it did influence the results, it was hardly ever substantial. Figure 4a–c shows that the effect of P_{ms} far exceeds the mobility effect. If anything can be concluded from these results, it is that low mobility does slightly decrease the potential invasion success.

Fitness

When the standard experiment, without differences in fitness, is compared with simulations assuming an increased fitness of hybrids, it turns out that the percentage of fixation of the invading allele clearly increases in the case of heterosis, whereas the opposite is true when the hybrids have a reduced fitness (Fig. 5). This predictable effect is most dramatic in a situation with a very low mating success; if that value is near 0, i.e. when mirror-image individuals cannot mate, a 10 per cent fitness increase brings the fixation percentage from 0.4 to 2.0 in a 16/4 simulation, whereas a 50 per cent increase lifts this percentage to 39.1.

Dominance of the chirality allele

The dominance or recessiveness of the chirality allele strongly affects the fixation percentages, at least in small populations with a relatively high number of invaders and a very low mating success. For example, for a mating success of 0, comparing dominance with recessiveness of the mutant allele, the simulations 16/4, 16/8 and 16/16 give the fixation percentages 2.2 vs. 0.4, 9.0 vs. 0.9 and 28.6 vs. 1.5, respectively. In these experiments, a uniform population is formed after 6, 8 and 13 generations, on average. With increasing P_{ms}, the effect of the domi-

nance of the mutant allele diminishes and the average number of generations required to get a uniform population increases (Fig. 6). With a mating success of 80 per cent, for example, the fixation percentages in the series of simulations mentioned before are 8.7 vs. 5.7, 16.4 vs. 11.1, and 27.4 vs. 17.1.

Discussion

Some authors (Gittenberger, 1988; Orr, 1991) have discussed the mechanism behind a shift in coiling direction in snails and have related such a shift to speciation. This is correct only as far as it concerns snails with globular shells, which are completely reproductively isolated if they are mirror-image and homozygous for coiling direction. In this paper, fixation of a mutant chirality allele is dealt with in the first place, irrespective of its consequences. If fixation happens in a population of snails with slender, more or less spindle-shaped shells (Fig. 8), and successful mating is hampered but still possible, full speciation, implying reproductive isolation, requires additional differences (resulting from for example a dramatic bottle-neck effect). Only for cases in which such differences did not evolve do we agree with Johnson et al. (1990, p. 464), that 'those populations ... are likely to be swamped upon secondary contact'. We disagree, however, when Johnson et al. (1990, p. 464) come to the conclusion that chirality 'is particularly unlikely to serve as the basis for sympatric speciation' in snails. In that conclusion concerning snails in general, the category of cases in which opposite coil results in full reproductive isolation (Fig. 7) is overlooked. Orr (1991, p. 768), while concluding that single-gene speciation on the basis of chirality is possible, 'in small isolated populations or among the founders of a new colony', refers to exactly that forgotten category.

In some highly exceptional cases, the genetics behind chirality might differ from what has been described so far. This applies for example to the speciose genus Alopia (see Grossu, 1981), with several mirror-image alleged sister species, suggesting the repetitive, thus easy, achievement of opposite coil and subsequent fixation in allopatric populations. Another special case might be found in species of Amphidromus, with populations containing both dextral and sinistral individuals in reasonable percentages (Laidlaw & Solem, 1961). It is unlikely that Amphidromus species simply represent an intermediate stage in chirality-based speciation, as Gittenberger (1988) suggested. Our simulations, starting from the generally accepted model with regard to chirality genetics, do not support a stable
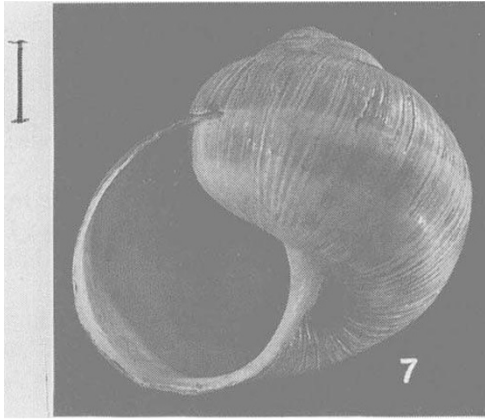
Fig. 7 An aberrant, sinistral specimen of *Helix pomatia* (colln. Nationaal Natuurhistorisch Museum, Leiden, the Netherlands), exemplifying globular shells; according to Meisenheimer (1912, p. 131), mirror-image individuals of this species fatigue one another for days and weeks in courtship, without achieving a final copulation ('tage- und wochenlang mühen sich die Tiere im Liebesspiel ab, ohne zur definitiven Begattung gelangen zu können'). Scale 1 cm.
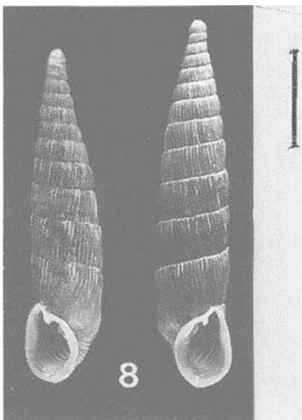
Fig. 8 Both a dextral and a sinistral specimen of *Balea biplicata* (colln. Nationaal Natuurhistorisch Museum, Leiden, the Netherlands), the former one from Degner's (1952) experimental populations; specimens with such shells can copulate. Scale 0.5 cm. Photographs: A.'t Hooft (R.U., Leiden).

situation as seems to occur in *Amphidromus*.

Our results show that Johnson *et al.* (1990) and Asami (1993) were right in assuming that for fixation of the mutant allele it is important whether this mutant allele is dominant or not. Dominance or recessiveness turned out to be of paramount relevance even for its occasional fixation. Unfortunately, this factor cannot easily be investigated in cases with a change in coiling direction in nature. Experimental

hybridization of sister species with opposite coiling directions may provide the relevant data here. These would be very difficult to obtain in practice, however, because at least an F_2 generation is required.

The optimal fixation scenario includes small populations, with few mutants (invaders), although the mutant allele will remain 'available' for a much longer time in large populations. According to Orr (1991, p. 768), the 'maternal inheritance .. appears to have the greatest effect on the likelihood of single-gene speciation'. Taking into account that this statement refers to only a subsample of the cases, we still have to emphasize the relevance of dominance or recessiveness, not considered by Orr. The maternal effect, which delays the negative consequences of the mutation, is instrumental too, but to a lesser degree. The mobility of the snails is of minor importance. Of course, heterosis would aid substantially in achieving fixation.

The fact that far more 'inverse taxa' occur among snails with slender shells is explained by the results of the simulation experiments, indicating that next to other factors, P_{ms}, the mating success (shell shape), is very important. We may assume that in most cases in evolutionary history, a mutant chirality allele entered the population in a situation with only a reduced mating success. The simulations show that if a change in coiling direction eventually followed in ana- or cladogenesis (in only a population at first), this resulted from a coincidence of very improbable circumstances. This might explain the obvious bias in coiling direction in gastropods (Robertson, 1993), still existing after many years of evolution. Coiling direction can switch, but not easily.

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