

The effect of chirality phenotype and genotype on the fecundity and viability of *Partula suturalis* and *Lymnaea stagnalis*: implications for the evolution of sinistral snails

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1 ABSTRACT: Why are sinistral snails so rare? Two main hypotheses are that selection acts
2 against the establishment of new coiling morphs, because dextral and sinistral snails have
3 trouble mating, or else, a developmental constraint prevents the establishment of sinistrals. We
4 therefore used an isolate of the snail *Lymnaea stagnalis*, in which sinistrals are rare, and
5 populations of *Partula suturalis*, in which sinistrals are common, as well as a mathematical
6 model, to understand the circumstances by which new morphs evolve. The main finding is that
7 the sinistral genotype is associated with reduced egg viability in *L. stagnalis*, but in *P. suturalis*
8 individuals of sinistral and dextral genotype appear equally fecund. As the strength of
9 frequency-dependent selection against the rare chiral morph in *P. suturalis* also operates over
10 a narrow range, the results suggest a model for chiral evolution in which constraints are
11 possible, but new sinistral alleles can sometimes be unconstrained. Since chirality or left-right
12 asymmetry does not vary in most other major taxonomic groups, the implication is that this is
13 either because of a constraint and/or because most taxa do not have conspicuous external
14 asymmetries upon which selection can act.

1 Introduction

2 Sinistrals make up much less than 10% of all snail species (Asami, 1993). Why are sinistral
3 snails so rare? The first and most obvious hypothesis is that since dextral and sinistral snails
4 have trouble mating, positive frequency-dependent selection prevents the establishment of new
5 sinistrals (Asami *et al.*, 1998). This is certainly correct in snails that mate in a ‘face-to-face’
6 position. As pairs of opposite coil are not at all able to mate, or do so only rarely (Asami *et al.*,
7 1998, Ueshima & Asami, 2003, Davison *et al.*, 2005b), new sinistrals will tend to lack mates
8 and eventually go extinct. A general problem with this hypothesis is that a large proportion of
9 snail species mate by ‘shell-mounting’, a position that usually allows matings between coils
10 (Johnson, 1982), so reducing selection against the novel type (Asami *et al.*, 1998). Because a
11 large section of snails can also self-fertilise, or fertilise externally (as can some marine
12 molluscs), a better hypothesis to explain the lack of sinistrals might be that there is stabilising
13 selection on dextrality (Vermeij, 1975). Development might constrain the establishment of new
14 sinistral morphs because of intrinsic genomic incompatibilities between the sinistral allele and a
15 dextral genetic background. An unfortunate problem with this theory, however, is a lack of
16 experimental or observational support, and an understanding of how a constraint may be
17 imposed. Moreover, while dextral-to-sinistral chiral evolution has been rare in snails, it has still
18 occurred repeatedly (Asami *et al.*, 1998), necessitating an explanation. An examination of
19 exceptional cases may therefore contribute to a general explanation of observed pattern.

20 Presently, there are two main theories to explain how new sinistral morphs evolve. The
21 first suggests that new chiral morphs become established by the random accumulation of
22 sinistral alleles, either through drift in small populations, through founder effects, or through
23 inbreeding arising from self-fertilisation. This idea has attracted particular attention because a
24 consequence of the maternal inheritance of chirality (explained below) is that “single-gene
25 speciation” in sympatry is theoretically possible (Gittenberger, 1988, Johnson *et al.*, 1990, Orr,
26 1991, Asami *et al.*, 1998, Stone & Björklund, 2002, Ueshima & Asami, 2003, Davison *et al.*,

1 2005a). The second theory suggests that selection is needed to establish new chiral morphs by
2 counterbalancing the mating disadvantage of the chiral minority. The oldest and most popular
3 view of how this selection occurs is through interactions between sympatric species. If a dextral
4 species coincides geographically with another, closely-related, dextral species, a sinistral
5 population can become established through reproductive character displacement (Clarke &
6 Murray, 1969, Murray & Clarke, 1980, Davison *et al.*, 2005a, Uit de Weerd *et al.*, 2006). More
7 recently, sexual selection (Schilthuizen *et al.*, 2007) and especially predation (Inoda *et al.*, 2003,
8 Dietl & Hendricks, 2006, Hosono *et al.*, 2007) have also been implicated in potentially giving
9 advantage to the rare morph.

10 Unfortunately, data that enable the testing of these two theories are limited and indirect.
11 We therefore set out to understand how sinistral snails evolve by examining crosses between
12 sinistral and dextral *Lymnaea stagnalis* pond snails, and by observing variation in natural
13 populations of terrestrial *Partula suturalis*. The main aim was to investigate evidence for a
14 developmental constraint by comparing the fitness of sinistral or dextral alleles in different
15 genetic backgrounds: if constraint is a direct consequence of an allele at the chirality locus
16 being deleterious, then 'fitness' ought to co-segregate with the allele; otherwise, if fitness is a
17 consequence of epistatic interactions involving the chirality genotype and other loci, then fitness
18 should be relative to the genetic background. Secondly, we were also able to use data from
19 *P. suturalis* to examine the mechanism by which frequency-dependent selection operates
20 against rare chirality morphs.

21 Together, the results may enable an understanding of the interactions at both the
22 genetic and phenotypic levels. Molluscs are exceptional, because chirality or 'left-right
23 asymmetry' is extraordinarily conserved across most metazoan phyla, and so does not usually
24 evolve. We therefore expect that the results should be relevant beyond molluscs, including
25 other spiralian phyla (annelids, echiurans, vestimentiferans, sipunculids, and nemertean) and
26 also vertebrates, because an understanding of variation in the former may give some clues to
27 explain the invariance of other groups. This is especially pertinent since it has very recently

1 been discovered that a gene, *nodal*, previously supposed to be confined to the deuterostomes,
2 is implicated in the expression of chirality in both molluscs and vertebrates, suggesting that
3 some of the present-day developmental pathways are an ancestral feature of the Bilateria
4 (Levin, 2005, Grande & Patel, 2008). Moreover, a general pattern that has emerged recently is
5 that variable genetic regulatory networks underpin many apparently conserved developmental
6 processes (Chouard, 2008). If the same turns out to be the case for left-right asymmetry in
7 general, then why is the left-right asymmetry phenotype canalised or 'buffered', except in
8 molluscs?

9 A crucial point in understanding the dynamics of chirality in molluscs is the maternal
10 inheritance - a single 'maternal effect' locus acts in the mother and determines the coil of her
11 offspring (Boycott & Diver, 1923, Sturtevant, 1923, Freeman & Lundelius, 1982, Schilthuizen &
12 Davison, 2005). This generational delay in the expression of the gene may result in rather
13 complicated population dynamics, as well as terminological confusion between phenotype and
14 genotype (Kirkpatrick & Lande, 1989, Lande & Kirkpatrick, 1990, Davison *et al.*, 2005a). For
15 clarity, we therefore refer to an individual's coil phenotype (indicating its mother's genotype) in
16 words (sinistral or sin, dextral or dex) and its genotype in italicised letters representing
17 alternative alleles (*S*, *D*). Also, while we appreciate that asymmetry can take many forms
18 (Palmer, 1996, Palmer, 2004), for simplicity, the scope of this work is largely restricted to
19 asymmetries of the entire body and shell, those that are maternally inherited and expressed
20 early in development.

21 Finally, a general scarcity of empirical data on chirality has arisen not only because
22 populations of snails that vary in their chirality are rare, but also because the maternal
23 inheritance means that it is laborious to infer genotype from juvenile phenotypes when all the
24 eggs that hatch from a female produce only a single data point. Therein lies one of the benefits
25 of using *P. suturalis* in particular. As the species is ovoviviparous, the coil of the intrauterine
26 young indicates the genotype of the mother, and in turn the mother's coil indicates the
27 genotype of its mother. *P. suturalis* is also unusual because purely sinistral populations are

1 common, separated from purely dextral populations by steep clines (Clarke & Murray, 1969; the
2 sinistral allele is dominant over the dextral allele). In contrast, *L. stagnalis* is a predominantly
3 dextral species in which sinistral individuals are rare (Asami *et al.*, 2008; dextral is dominant
4 over sinistral).

5

1 **Materials and Methods**

2 **The snails**

3 *L. stagnalis* is a fully self-fertile hermaphrodite; sinistrals and dextrals are able to mate with
4 each other, albeit with some modifications of behaviour.

5 The sinistral stock of *L. stagnalis* used in this experiment was donated by Joris Koene,
6 having been maintained within the laboratory for some years, and is ultimately derived from the
7 source of Asami *et al.* (2008). The dextral stock was taken from a pond in the University of
8 Nottingham in 2005. The stock had gone through ~5 generations in the laboratory before the
9 experiment began. For this experiment, the snails were kept in aerated 12-litre plastic tanks
10 and fed *ad libitum* with lettuce, changing the water about once per month (Thomas, 1986). To
11 maximise the throughput and to reduce the time to maturity, up to 20 snails of the same mother
12 were kept in each tank, being grown from egg to sexually mature adult. To score each snail for
13 its chirality genotype (see below), two snails were placed in a tank, kept apart by a net. When
14 young snails were near to hatching, egg capsules (containing between 1 and 200 eggs) were
15 removed, and the chirality of the developing young was determined under a dissecting
16 microscope.

17 Like *L. stagnalis*, *P. suturalis* is a hermaphrodite, but it rarely self-fertilises. In any single
18 mating, an individual takes either the male or female role. Sinistrals and dextrals are able to
19 mate, as before, with behavioural modifications and at a much lower frequency than with snails
20 of the same coil (Johnson, 1982).

21 The samples of *P. suturalis* came from several collecting trips made by B.C. and Jim
22 Murray to the island of Moorea, French Polynesia in the 1960s. On Moorea, purely sinistral and
23 purely dextral populations of *P. suturalis* are separated by steep clines of transition, ~0.5 km to
24 1 km wide (Johnson *et al.*, 1993). The samples used in the present study came from 32
25 populations (906 individual sinistral snails and 621 dextrals), which were collected in 1962 and
26 1967 from different locations near Mount Mouaroa. Each sample was restricted to a 10 x 10

1 metre square (the detailed distributions of chiral morphs in this region are illustrated in Clarke &
2 Murray, 1969). A technician recorded the chirality of individual, wild-collected snails, then
3 dissected out intrauterine young, and recorded their phenotypes, so as to infer the genotypes of
4 the mothers (see below; raw data is in Supplementary Table 1).

5 **Genetics**

6 Since dextrality is dominant in *Lymnaea*, only five phenotype/genotype combinations are
7 possible. Dextral snails are of genotype *DD*, *DS* or *SS*, whereas sinistral snails are of genotype
8 *DS* or *SS*. Dex *DD* and dex *DS* are indistinguishable because they both produce dextral
9 offspring; sin *DD* snails should not exist, except as rare aberrations, because the mother would
10 have carried the dominant dextral allele. In *P. suturalis* the sinistral allele is *dominant* over the
11 dextral. Once more, five combinations of maternal and own genotypes are possible, but they
12 differ from those in *Lymnaea*. They are sin *DS*, sin *SS*, sin *DD*, dex *DS* and dex *DD*. Sin *DS*
13 and sin *SS* are indistinguishable because they both produce sinistral offspring. Dextral *SS*
14 homozygotes should not exist, except as rare aberrations, because the mother would have to
15 carry the dominant sinistral allele.

16 **Crosses**

17 In many snail genera, sinistral and dextral individuals are unable to mate, so genetic
18 experiments are not possible. In *L. stagnalis*, mating between coils occurs, but at a lower
19 frequency than matings within coils and involves some modifications of behaviour. Although the
20 species is hermaphrodite, individuals preferentially outcross, but will fertilise themselves if kept
21 in isolation (Puurtilinen *et al.*, 2007).

22 Using a method similar to that of Hosoiri *et al.* (2003), we crossed virgin dextral
23 (genotype *DD*) and sinistral (*SS*) snails to create a sinistral heterozygous F₁ generation (*DS*)
24 (Fig. 1). To distinguish F₁ hybrid snails (*DS*) from the offspring of self-fertilising sinistrals (*SS*),
25 the progeny of the sinistral mother (all themselves sinistral) were raised to adulthood, and the
26 shell phenotype of their babies checked. F₁ hybrids (genotype *DS*) produce dextral babies,

1 whereas if the maternal parent self-fertilised, then the supposed “F₁” will be of genotype SS,
2 and so produce sinistral babies (Fig. 1). Eggs from individual F₁ hybrids were then raised to
3 adulthood, allowed to cross with one another, so creating an F₂ generation. To determine the
4 genotype of the F₂ individuals, the phenotype of their babies was scored (dextral babies being
5 *DD* or *DS*; sinistral babies being *SS*). Virgin F₂ snails that produced dextral babies were allowed
6 to self-fertilise, their virgin F₃ offspring were raised, self-fertilised, and the coils of the F₄
7 offspring were scored. By this means, we were able to distinguish F₂ *DS* individuals from F₂ *DD*
8 individuals, so that segregation at the chirality locus could be followed into the F₃ generation.

9 **Proxies of ‘fitness’**

10 The viability of *L. stagnalis* offspring was scored at the same time as chirality. The first egg
11 capsule from each individual snail was dissected in the few days before hatching, and the
12 number of living, normally-developed snails was counted (abnormal embryos arrest at all
13 stages, or have gross morphological deformities, so they are easy to recognise; see Fig. 2). To
14 test the reproducibility of these ‘fitness’ statistics, a second capsule was removed from a subset
15 of individual snails and the procedure repeated. To establish whether any reduction in offspring
16 viability is a direct consequence of the chirality locus, the offspring viability of F₁, F₂ and F₃
17 snails was checked, the aim being to determine if this character segregates with the chirality
18 locus. The crucial point is that following recombination during meiosis, individual F₂/F₃ snails of
19 different chirality genotypes (*DD* or *DS* versus *SS*) will on average only differ at the chirality
20 locus, or in genes tightly linked to it. Thus, any viability differences can be ascribed only to the
21 chirality haplotypes.

22 For *P. suturalis*, a different but related measure of ‘fitness’ was calculated. On dissection,
23 the numbers of intrauterine unhatched eggs and hatched young were counted for each adult,
24 wild-collected snail (its ‘fecundity’). The total numbers of offspring were then counted, and the
25 proportion of hatched individuals was calculated for each maternal phenotype or genotype.

1 Despite the oddities of maternal inheritance (Kirkpatrick & Lande, 1989, Lande &
2 Kirkpatrick, 1990), strong associations still tend to occur between phenotype and genotype,
3 with the result that some phenotype-genotype combinations are rare. To mitigate this problem,
4 it was sometimes necessary to pool data across populations of *P. suturalis*, and to control for
5 inter-population variation by always including the same number of randomly-chosen individuals
6 of each kind of snail from each population.

7 **Mathematical model**

8 In earlier work we used a mathematical model to predict the expected proportions of sinistral
9 snails amongst the offspring of sinistrals, according to the frequency of the sinistrals, under the
10 assumption that there is no selection (Figure 6 in Davison *et al.*, 2005a). This was done
11 because the expected proportions of each offspring phenotype, and the frequency of each
12 coiling morph within the population, represent data that can be gathered in the field, and
13 because they have predictive value. A third parameter, α , describes the degree of interchiral
14 mating, where $\alpha = 0$ indicates random mating between coiling types and $\alpha = 1$ indicates no
15 interchiral mating at all. The derivation of the model is explained in Davison *et al.* (2005a). Here,
16 the model is extended to include the expected proportions of dextral snails amongst the
17 offspring of dextrals, according to the frequency of the latter. For the first time, the *Partula* data
18 enabled a comparison between theoretical predictions and those found in nature. Deviations
19 from expectation might help to understand the dynamics of chiral evolution.

20

1 Results

2 *Lymnaea stagnalis*

3 The viability of offspring in egg capsules from 648 individual snails was assessed, counting and
4 checking 49835 eggs in the process. Homozygous sinistral individuals of the laboratory stock
5 (all genotype *SS*; the F_0) of the pond snail *Lymnaea stagnalis* were considerably less fit than
6 the homozygous dextral stock (*DD*). On average, only about 62% of offspring from individual
7 sinistral snails were viable, compared with 96% in dextral stocks (Fig. 3a). In the sinistral stock,
8 development arrested at all stages from the first few cell divisions to just before hatching, often
9 producing young snails with grossly malformed shells (Fig. 2).

10 A significant proportion of the viability or 'fitness' differences are likely to be due to a
11 maternal effect of the chirality locus itself (or of linked genes) because lower viability
12 segregated with the sinistral alleles in crosses (Table 1; Fig. 3). First, it was found that sinistral
13 F_1 snails of genotype *DS* are of comparable viability to the dextral stock snails of genotype *DD*,
14 presumably a consequence of the dominant dextral allele (97% offspring viability, similar to the
15 F_0 dextral stock). Second, viability varied markedly between genetically dextral or sinistral
16 snails in the F_2 and the F_3 . F_2 snails that were genetically sinistral (*SS*) had a hatch rate of 43%
17 compared with 88% for dextral F_2 snails (genotype *DD/DS*) (Table 1). In the next generation,
18 the F_3 , the differences were of the same magnitude, 92% and 47% respectively.

19 There was also some evidence for epistasis, implying that offspring viability may be
20 further reduced because of an interaction between the chirality allele (dextral or sinistral) and
21 the genetic background. Genetically sinistral F_2 and F_3 snails that inherited part of their genome
22 from a dextral lab stock were markedly less fit (43%, 47% viability) compared with genetically
23 sinistral snails from a purely sinistral background (62%). The range of observable viabilities was
24 also greater (F_2 : 2% to 97%; F_3 : 1% to 82%, compared with F_0 : 33% to 94%) (Fig. 3). Similar,
25 though less striking results were obtained for the dextrals: genetic dextral F_2 and F_3 snails were
26 less fit (88%, 92% viability) compared with genetically dextral snails from the dextral stock

1 (96%), the former also having a greater range (F_2 : 9% to 100%; F_3 : 48% to 1%; compared with
2 F_0 : 71% to 100%).

3 For a subset of the snails, the offspring viability was measured in two separate capsules.
4 A strong correlation was found (Fig. 4), implying that although a common environment probably
5 has a role, it is likely that there is also a significant maternal genetic element in offspring
6 viability.

7 ***Partula suturalis***

8 We first assessed the 'fecundity' of sinistral and dextral *P. suturalis*. Overall, no major
9 differences or trends were detected, regardless of whether snails of different coiling phenotype
10 or genotype were compared, using either the total number of offspring, or the proportion
11 hatched. By phenotype (Table 3), dextrals had a slightly greater hatch rate in 12/22 cases, but
12 a lower hatch rate in 10, with one comparison being individually significant (population 455).
13 Summing across all the results, the hatch rate of eggs within dextral-coiling individuals was
14 50.4% ($\pm 2.8\%$) and the hatch rate of snails within sinistral individuals was 47.3% ($\pm 2.2\%$), not
15 significantly different from one another.

16 Though fewer comparisons were possible, the same result was found when comparing
17 genotypes (Table 3). Dextral coiling snails with a dextral genotype (*DD*; mean hatching rate
18 from pooled data = 38% ± 2.6) produced offspring with the same hatch rate as dextral snails
19 with a sinistral genotype (*DS*; mean = 38% ± 2.7). Similarly, sinistral snails with a dextral
20 genotype (*DD*; mean from pooled data = 41% ± 2.4) produced offspring with nearly the same
21 hatch rate as dextrals with a sinistral genotype (*SS* or *DS*; mean = 42% ± 2.5). Neither were
22 there differences in the total number of offspring produced (Tables 2, 3).

23 For the second part of the analysis, we compared the total number of offspring, or
24 'fecundity', according to the percent of each coil morph in a population. If positive frequency
25 dependent selection operates against the rare morphs, the expectation is that the common

1 morph will produce more offspring. No evidence was found for this expectation (Fig. 5). The
2 'fecundity' of dextrals was high even when the frequency of sinistrals was > 97% within a
3 population (population 121, 97.9% sinistral, mean number of offspring dextral and sinistral = 2.5,
4 2.1; population 124, 97.3% sinistral, mean number of offspring dextral and sinistral 2.5, 1.9;
5 population 125, 97.2%, mean number of offspring dextral and sinistral 2.0, 2.1).

6 For the third part of the analysis, we used an extension of a mathematical model
7 (Davison *et al.* 2005a, notation is the same here) to predict the proportions of each coil in the
8 offspring, according to the phenotype of the mother and the overall frequency of the
9 phenotypes in the population (Fig. 6). At equilibrium, the proportion of sinistral offspring from
10 sinistrals mothers should be $(2sP-2w)/s$; the proportion of dextrals from dextral mothers is $1-$
11 $2P+2w/d$ (see Supplementary method for full explanation). The empirical data (Supplementary
12 Table 2) are in line with expectation when sinistral *P. suturalis* are common (> 50%; Fig. 6).
13 However, when the proportion of sinistrals was less than 50%, sinistral offspring were rarer
14 than expected. The explanation could be due to sampling variation, or the system was not at
15 equilibrium / perturbed by selection. For dextrals the situation was similar, though less
16 obviously so. There were generally fewer dextral offspring than expected when dextrals were
17 the minority (< 50%), and a slight excess when they were the majority. The same explanations
18 and caveats must apply.

19

1 Discussion

2 'Fitness' effects of sinistral alleles

3 The findings reported here suggest that the *S* haplotype of *L. stagnalis* has both direct and
4 indirect 'fitness' effects. Genetically sinistral *L. stagnalis* are inherently less fit in a direct sense
5 because of the malfunctioning or absence of some factor in the eggs that ~halves the number
6 of young snails when compared with genetic dextrals. Sinistrals also appear to be less fit in an
7 indirect sense, because the chirality haplotypes may further affect viability epistatically with
8 alleles at other loci, whether in the conventional Mendelian manner, or maternally. The main
9 case for epistasis is the observation that genetically sinistral F_2 and F_3 snails that had inherited
10 part of their genome from the dextral stock were markedly less fit, because they produced
11 offspring with a greater range of viabilities, compared with genetically sinistral snails from a
12 purely sinistral background (Fig. 3).

13 One other possibility that can not be discounted is that the viability of *L. stagnalis* eggs in
14 the sinistral stock is reduced because of inbreeding, and is alleviated in the F_1 (Table 1)
15 because of heterosis. However, there is no inherent expectation that genetically dextral (DD or
16 DS) or sinistral (SS) F_2 and F_3 snails should differ in the viability of their eggs, unless the genes
17 that determine heterosis are linked to the chirality locus. It is simpler to invoke the chirality locus
18 itself as the major cause of inviability, rather than the action of unknown linked loci.

19 In contrast, no differences in the total number of offspring, or the proportion of hatched
20 eggs, were found between dextral or sinistral *P. suturalis*. Moreover, no differences in our
21 measures of relative fitness were found between genetically dextral or sinistral *P. suturalis*.
22 Although an absence of evidence is not proof, and many other differences separate the two
23 species (including dominance of chirality alleles), the results make sense in terms of the
24 frequencies of chiral morphs in the wild. Sinistral *L. stagnalis* are extremely rare, and almost
25 invariably form a tiny minority of individuals (Asami *et al.*, 2008). In contrast, sinistral *P.*
26 *suturalis* are (or were, before they became functionally extinct; Coote & Loeve, 2003) the

1 predominant morph across large regions of Moorea, with dextral and sinistral populations
2 separated by sharp clines, associated with the presence or absence of other species.

3 **A model for the evolution and action of sinistral alleles**

4 Since the chirality gene controls a part of the conserved cleavage programme that is found in
5 most spiralian (Henry & Martindale, 1999), it is not surprising from the consideration of
6 developmental genetics that a mutation should have fitness consequences in *Lymnaea*. In fact,
7 the more surprising observation is that it is possible to alternate chirality alleles in *Partula*
8 without an evident effect on a measure of 'fecundity'. One explanation can be largely ruled out.
9 It is inconceivable that epistatically interacting alleles in linkage disequilibrium, as a result of
10 restricted gene-flow, might maintain fitness unless they are closely linked, because the
11 mathematical model indicates almost completely free gene-flow between the two morphs
12 (Davison *et al.*, 2005a). We therefore suggest that the majority of new sinistral alleles are
13 deleterious in a dextral genetic background, but a minority are nearly neutral with respect to
14 'fecundity'. The successful minority of sinistral alleles have equivalent 'fecundities' in both
15 dextral and sinistral backgrounds, so that for *Partula* a developmental constraint (Vermeij,
16 1975) does not seem to exist.

17 If the model is correct it raises the question of how neutrality is possible, given that
18 sinistral/dextral chirality alleles probably interact with the products of many other loci. In the
19 hypothetical view of Brown and Wolpert (1990), chirality is determined by a pre-existing
20 asymmetric molecular reference: an asymmetric gradient is created if an 'F-molecule' aligns
21 with anterior-posterior and dorsal-ventral axes, so transporting an effector molecule towards the
22 left or right. Asymmetry is thus entirely dependent upon the chirality (and subsequent
23 alignment) of the F-molecule. The implication for the work here is that mirror-image snails with
24 differing chirality alleles may be equally fit because the only difference between them is the
25 chiral 'F-molecule' that sets up the asymmetry in the first place (Brown & Wolpert, 1990). As
26 long as this molecule interacts equivalently with others, development should occur as normal,

1 because the same set of instructions may produce both chiral forms. McManus (2002, p96)
2 provides an explanatory analogy: if two persons are standing back-to-back on the Greenwich
3 meridian line, and each follows the same set of shouted instructions (“North”, “South”, “away
4 (from the meridian)” or “towards (the meridian)”) then their walks will be a mirror image.
5 Although the analogy is appealing, its molecular equivalent is elusive.

6 The results of this study may well be relevant more widely. Earlier data support the view
7 that the relative fitness is reduced in sinistral *L. peregra* (Boycott *et al.* 1930), although in this
8 work the segregation and fitness's of the coiling alleles were not investigated. Further
9 supportive evidence, albeit less rigorous, comes from some other species (Gause &
10 Smaragdova, 1940, references in Gould *et al.*, 1985). In *Partula*, chirality alleles or linked loci
11 certainly have pleiotropic effects on shell shape (Crampton, 1932, Johnson, 1987). In *Cerion*
12 rare sinistral individuals are not exact mirror images of dextrals, and such changes may
13 indicate pleiotropic effects of the chirality locus (Gould *et al.*, 1985). In *L. stagnalis*, early
14 cleavage events in dextrals are not a mirror image of development in sinistrals (Shibazaki *et al.*,
15 2004, Wandelt & Nagy, 2004). Entirely sinistral species of *Lymnaea* have existed (Pierce,
16 1996), and purely sinistral populations of *Lymnaea* have been reported (Zimmerman, 1948),
17 indicating that chiral evolution does occur in this genus, and is not just an aberration. Other
18 genera of land and freshwater pulmonates are largely or entirely sinistral (for example *Clausilia*
19 and *Physa*). Finally, *Amphidromus* is likely to be a particularly useful genus in understanding
20 chirality, and the potential for associated fitness effects, since sinistral and dextral morphs are
21 apparently co-maintained by negative frequency-dependent selection (Schilthuizen *et al.*, 2007,
22 Sutcharit *et al.*, 2007).

23 **Positive frequency-dependent selection**

24 It is sometimes assumed that new chiral morphs of snails evolve only rarely because
25 individuals of the opposite sort are less likely, or even completely unable, to find a mate. This
26 explanation is not particularly satisfactory because many snails, especially high-spined ones like

1 *Lymnaea* and *Partula* (Asami *et al.*, 1998), are able to mate with other chiral morphs, and also
2 self-fertilise. As there has been an absence of data on which to test hypotheses, the data for *P.*
3 *suturalis* are useful because they are able to shed new light on the problem.

4 Johnson (1982) showed experimentally that in *P. suturalis* pairs of opposite coil given
5 no other choice of partner courted each other at the same frequency as pairs of the same coil,
6 but succeeded in copulation only about 20% as often. They produce about 60 to 70% fewer
7 young. Johnson also found that the fertilities of dextrals and sinistrals were the same when
8 taken from a population with equal frequencies of the two morphs, whereas among those taken
9 from a population in which sinistrals occurred at low frequency, the rare sinistrals produced
10 fewer young than the common dextrals. These observations are *prima facie* evidence for
11 frequency-dependent selection against the rare morph. Our present data instead indicate that
12 in natural populations the number of young within dextral mothers is as high as it is within
13 sinistrals, even when the proportion of sinistrals is > 97% of the population. They imply that rare
14 individuals are still able to gain sufficient matings to fertilise their eggs.

15 The discrepancy between our results and Johnson's is almost certainly because
16 Johnson measured fecundities over a long period in the laboratory, more than a year after
17 mating or collection from the wild. He was thus observing the results of long-term sperm
18 storage, which in *P. suturalis* can last as long as two years. Our results represent the
19 consequences of mating in the short term. The discrepancy is also illuminating. Sperm-storage
20 becomes selectively important when meeting and mating is infrequent, as it is when population
21 densities are low. Thus the strength of the positive frequency-dependence may be negatively
22 density-dependent. The samples reported here were taken from dense populations, in which
23 encounters would be very frequent, so that even rare morphs would find male partners of one
24 coil or another.

25 Comparisons between the empirical frequencies of chiral morphs *in utero* and
26 predictions from our mathematical model may also suggest a role for frequency-dependent
27 selection. When sinistrals are rare the frequency of sinistral offspring within sinistral snails is

1 less than expected, with similar results for dextrals (Fig. 6). A considerable caveat, however, is
2 that it is not possible to be certain that the departures are greater than expected due to
3 sampling variation, or the populations not being at equilibrium. Taking all of the inferences
4 together (Figs. 5, 6), however, the best explanation is that individuals of the rare morph have
5 more difficulty in mating productively, but are nearly always able to gain sufficient sperm to
6 fertilise their eggs, at least for the short term. As many of these mates will be of the opposite
7 chirality, so tend to have a matching chirality allele genotype, then subsequent generations will
8 be more likely to produce snails of the opposite chirality.

9 The overall conclusion must be that positive frequency-dependent selection is often an
10 important factor in preventing the evolution of sinistral snails when snails are outcrossing and
11 have internal fertilisation. Nonetheless, the mode of action is more subtle than previously
12 supposed, and in particular, selection against the rare morph may be relaxed in high density
13 populations. In other circumstances, such as when opposite coil morphs are completely unable
14 to mate, sinistrals are most likely to evolve if a counter-balancing selection is also in operation,
15 such as character displacement (Clarke & Murray, 1969, Davison *et al.*, 2005a, Uit de Weerd *et*
16 *al.*, 2006), sexual selection (Schilthuizen *et al.*, 2007) or predation (Inoda *et al.*, 2003, Dietl &
17 Hendricks, 2006, Hosono *et al.*, 2007).

18 **The evolution of sinistral spiralian**

19 It is surprising that the establishment and evolution of chirality in other phyla has rarely been
20 considered, even though the Mollusca are only one of several taxa in the superphylum Spiralia
21 (Henry & Martindale, 1999). Part of the problem may be ascertainment, since sinistral snails
22 are much easier to identify than sinistral worms, but a survey of the historic literature carried out
23 by one of us (AD) failed to find any true sinistral-cleaving annelids (but see below). There
24 seems to be a real lack of sinistrals in other spiralian groups (Anderson, 1973, Kume & Dan,
25 1988), creating a paradox. Many sinistral snails exist, despite a possible mating disadvantage,
26 yet sinistral annelids, for example, are not known, even though they lack conspicuous external

1 left-right asymmetries and can be broadcast-spawning (hence having no problems of symmetry
2 in mating) (Anderson, 1973, Kume & Dan, 1988). As an explanation in terms of positive
3 frequency-dependence is not obviously tenable in these many spiralian, an explanation for the
4 difference between gastropod molluscs and others must lie instead in the nature of the
5 phenotype produced by of the chirality locus, perhaps combined with the existence of
6 developmental constraint. New sinistrals have evolved in snails precisely because of their
7 outward asymmetry, upon which extrinsic counter-selection can act (Clarke & Murray, 1969,
8 Murray & Clarke, 1980, Davison *et al.*, 2005a, Schilthuizen *et al.*, 2007). Sinistral annelids, and
9 other spiralian, would generally have failed to evolve because mutations of the chirality locus
10 were deleterious, but also because extrinsic selection was not usually asymmetric. The most
11 likely candidates for sinistrally-developing annelids, would be the minority of species with
12 conspicuous external asymmetries (e.g. the sinistral or dextral snail-like shells of some marine
13 worms; Palmer, 1996). A recent record of the first sinistral-cleaving annelid is therefore
14 significant because the species, a serpulid tube worm, has an external shell (Arenas-Mena,
15 2007).

16 In summary, the results suggest a model for chiral evolution in which constraints are
17 possible, but new sinistral alleles can also be unconstrained. Although the precise role of
18 frequency-dependent selection remains to be confirmed and clarified, especially whether it is
19 density dependent, our suggestion is that external asymmetry is a key to the evolution of new
20 chiral morphs. Without asymmetry, there is nothing upon which selection can act (e.g. Clarke &
21 Murray, 1969, Inoda *et al.*, 2003, Dietl & Hendricks, 2006, Hoso *et al.*, 2007, Schilthuizen *et al.*,
22 2007), so change (at best) depends upon random genetic drift and (at worst) is entirely
23 impeded by a developmental constraint. Since left-right asymmetry does not vary in most major
24 taxonomic groups, yet the *nodal* pathway is apparently an ancestral feature of the Bilateria
25 (Grande & Patel, 2008), then snails may be crucial towards the understanding this invariance.

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Table 1 Summary of egg viability counts for *L. stagnalis*. Each count is the proportion of viable snails in one capsule from an individual mother.

	F0		F1	F2	F3		
Phenotype	dextral	sinistral	sinistral	dextral	dextral	dextral	dextral
Genotype	<i>DD</i>	<i>SS</i>	<i>DS</i>	<i>DD</i> or <i>DS</i>	<i>SS</i>	<i>DD</i> or <i>DS</i>	<i>SS</i>
	n=46	n=38	n=22	n=285	n=90	n=115	n=52
Mean	0.96	0.62	0.97	0.88	0.43	0.92	0.47
SE	0.007	0.027	0.008	0.010	0.022	0.008	0.032
95% confidence limits	0.94 to 0.97	0.56 to 0.67	0.88 to 1.00	0.86 to 0.90	0.38 to 0.47	0.90 to 0.93	0.40 to 0.53
Range	0.71 to 1	0.33 to 0.94	0.88 to 1	0.09 to 1	0.02 to 0.97	0.48 to 1	0.01 to 0.82

Table 2 Summary of 'fecundity' for **phenotypically** dextral and sinistral coiling *P. suturalis*. The 'total offspring' count includes both eggs and hatched juveniles; the 'proportion hatched' is the number of hatched juveniles divided by the total. * indicates significant difference by two tailed t-test, $P < 0.05$; **, $P < 0.01$.

Site		Total offspring				Proportion hatched			
		N	Mean	SE	P	N	Mean	SE	P
120	dex	7	2.71	0.61		6	0.48	0.14	
	sin	94	2.94	0.14	0.680	85	0.53	0.03	0.643
126	dex	5	3.20	0.20		5	0.57	0.07	
	sin	29	2.28	0.24	0.131	24	0.45	0.04	0.259
127	dex	5	2.00	0.32		5	0.83	0.11	
	sin	8	2.25	0.41	0.676	8	0.58	0.11	0.149
128	dex	25	2.04	0.28		20	0.61	0.07	
	sin	27	2.63	0.23	0.110	24	0.50	0.05	0.202
129	dex	12	1.58	0.31	*	10	0.30	0.10	
	sin	7	2.86	0.26	0.013	7	0.54	0.06	0.097
130	dex	32	2.91	0.25		30	0.54	0.05	
	sin	13	2.31	0.38	0.198	11	0.56	0.08	0.820
131	dex	29	2.31	0.20		28	0.76	0.05	
	sin	15	1.67	0.29	0.069	12	0.57	0.10	0.070
132	dex	61	2.21	0.16		52	0.47	0.04	
	sin	42	2.40	0.17	0.423	39	0.57	0.04	0.062
196	dex	7	2.86	0.46		7	0.51	0.09	
	sin	65	2.80	0.17	0.917	58	0.32	0.03	0.026
274	dex	8	2.88	0.30		8	0.54	0.08	
	sin	50	2.66	0.16	0.611	46	0.57	0.03	0.657
277	dex	16	2.81	0.45		13	0.57	0.06	
	sin	28	3.04	0.25	0.639	27	0.52	0.03	0.523
375	dex	25	2.92	0.26		23	0.46	0.04	
	sin	12	3.17	0.46	0.616	11	0.53	0.07	0.318
376	dex	22	2.27	0.30		18	0.50	0.05	
	sin	14	2.86	0.27	0.184	14	0.47	0.07	0.717
439	dex	6	2.67	0.80		5	0.28	0.08	
	sin	11	2.64	0.45	0.972	10	0.50	0.09	0.138
440	dex	12	2.58	0.36		11	0.37	0.05	
	sin	25	2.56	0.28	0.961	23	0.41	0.06	0.691
444	dex	5	2.80	0.37		5	0.43	0.04	
	sin	44	2.52	0.18	0.611	41	0.33	0.03	0.195
453	dex	43	1.79	0.16		38	0.51	0.05	
	sin	28	1.64	0.19	0.557	24	0.56	0.07	0.562
454	dex	29	0.90	0.21		14	0.44	0.11	
	sin	29	1.41	0.23	0.104	20	0.44	0.08	0.982
455	dex	21	2.19	0.31	**	17	0.43	0.07	*
	sin	13	0.77	0.26	0.003	6	0.17	0.11	0.050
456	dex	11	1.55	0.49		7	0.63	0.13	
	sin	5	1.40	0.87	0.878	2	0.46	0.21	0.553
457	dex	19	2.11	0.27		16	0.37	0.06	
	sin	16	2.44	0.40	0.486	14	0.40	0.10	0.791
458	dex	30	1.53	0.17		26	0.49	0.07	
	sin	27	1.22	0.19	0.225	18	0.42	0.08	0.529

Table 3 Summary of 'fecundity' for **genetically** dextral and sinistral coiling *P. suturalis*. The 'total offspring' count includes both eggs and hatched juveniles; the 'proportion hatched' is the number of hatched juveniles divided by the total. * indicates significant difference by two tailed t-test, $P < 0.05$.

Site		Total offspring			<i>P</i>	Proportion hatched			<i>P</i>
		N	Mean	SE		N	Mean	SE	
132	dex <i>DD</i>	33	2.58	0.14	0.760	33	0.39	0.03	0.415
	dex <i>SD</i>	15	2.67	0.32		14	0.45	0.07	
455	dex <i>DD</i>	22	2.41	0.17	0.112	22	0.41	0.04	*
	dex <i>SD</i>	7	1.86	0.26		7	0.19	0.09	
457	dex <i>DD</i>	7	2.71	0.18	0.593	7	0.33	0.06	0.020
	dex <i>SD</i>	8	2.50	0.33		8	0.32	0.08	
457	dex <i>DD</i>	13	2.08	0.21	0.523	13	0.29	0.06	0.924
	dex <i>SD</i>	6	1.83	0.31		6	0.31	0.10	
Controlled pool	dex <i>DD</i>	66	2.76	0.12	1.000	66	0.38	0.03	0.925
	dex <i>SD</i>	66	2.76	0.13		65	0.38	0.03	
120	sin <i>DD</i>	6	2.83	0.17	0.049	6	0.42	0.06	0.522
	sin <i>SS/SD</i>	65	3.51	0.10		65	0.46	0.02	
130	sin <i>DD</i>	5	2.80	0.20	0.840	5	0.50	0.07	0.493
	sin <i>SS/SD</i>	6	2.67	0.56		6	0.61	0.13	
132	sin <i>DD</i>	7	2.71	0.42	0.864	7	0.57	0.08	0.447
	sin <i>SS/SD</i>	27	2.78	0.15		27	0.49	0.05	
277	sin <i>DD</i>	5	3.80	0.37	0.271	5	0.48	0.06	0.902
	sin <i>SS/SD</i>	20	3.20	0.25		20	0.49	0.02	
444	sin <i>DD</i>	8	2.88	0.23	0.596	8	0.34	0.07	0.767
	sin <i>SS/SD</i>	33	2.67	0.18		33	0.32	0.03	
Controlled pool	sin <i>DD</i>	67	3.01	0.12	0.445	67	0.41	0.02	0.692
	sin <i>SS/SD</i>	67	2.88	0.13		67	0.42	0.03	

Fig. 1 The crossing strategy. Sinistral snail (genotype SS) crossed to dextral snail (genotype DD), and sinistral F₁ offspring of sinistral mother raised to adulthood. In case of self-fertilisation, the phenotype of offspring raised from the F₁ was scored: true hybrids (genotype DS) produce dextral offspring, whereas selfed individuals (genotype SS) produce sinistral offspring (not used, hence the grey shading). Having recognised F₁ hybrids, snails crossed amongst themselves and F₂ offspring raised to adulthood (all dextral) and the phenotype/offspring viability of their young scored. F₂ snails are of genotype DD, DS (dextral offspring) or SS (sinistral offspring). F₂ SS offspring were used no more (grey shading), with the self-fertilised offspring of F₂ DD/DS raised to adulthood to form the F₃. DS heterozygotes from the F₂ generation were then retrospectively identified, by virtue of the fact that their offspring (F₃) must contain ~1/4 SS homozygotes.

Fig. 2 Abnormal and normal *L. stagnalis* juvenile snails from the same homozygous sinistral (SS) mother. The shell of the left hand snail does not have any obvious coil or chirality, compared with the anticlockwise twist of the right hand snail. Width of the egg capsule is approximately 1 mm.

Fig. 3 Proportion of viable *L. stagnalis* offspring produced by genetic dextral and sinistral snails in generation F₀, F₂ and F₃. In the F₁ generation, fitness of DS heterozygotes was equal to that of DD homozygotes (Table 1).

Fig. 4 For a subset of *L. stagnalis* snails, the offspring viability was measured in two separate capsules. For F₂ snails that are genetically sinistral (SS), the correlation between count 1 and 2 explains 64% of the variation; for genetic dextrals (DD, DS), the correlation explains 72% of the variation. Thus, while a common environment probably explains a proportion of this correlation, it is likely that there is a significant maternal genetic element to offspring viability, in addition to that caused by the chirality locus.

Fig. 5 The mean number of *in utero* offspring in dextral and sinistral *P. suturalis*, plotted according to the percent frequency of sinistrals in each population (\pm SE). The fecundity of

dextrals was equal to that of sinistrals, even when the latter are very common (> 97%). There were too few data to reliably estimate the fecundity of snails when sinistrals are rare.

Fig. 6 The expected proportions (area between lines) of each offspring phenotype according to whether the mother is sinistral (top) or dextral (bottom) coiling, under the assumption that there is no selection. The lines are the boundaries defined by extreme values of α , the parameter that describes the degree of interchiral mating, with the space between representing intermediate values of alpha (lower curve = random mating between chiral morphs; upper curve = no interchiral mating). The points are empirical data for *P. suturalis*, with a minimum sample size of five adults. In calculating the dependent variable, all the offspring from a single adult were treated as a single data point. See text for further explanation and interpretation; the model is explained in detail in Davison *et al.* (2005a).

Supplementary information. The extension of the mathematical model (Davison *et al.*, 2005a) to include the proportion of dextral snails amongst the offspring of dextrals against the frequency of dextrals within the population (see Fig. 6). References within the text refer to figures and equations from Davison *et al.* 2005a. File is provided in Mathematica and pdf formats.

Supplementary Table 1. The raw *Partula* data set.

Supplementary Table 2. The empirical data used for the plots in Fig. 6

Fig. 1

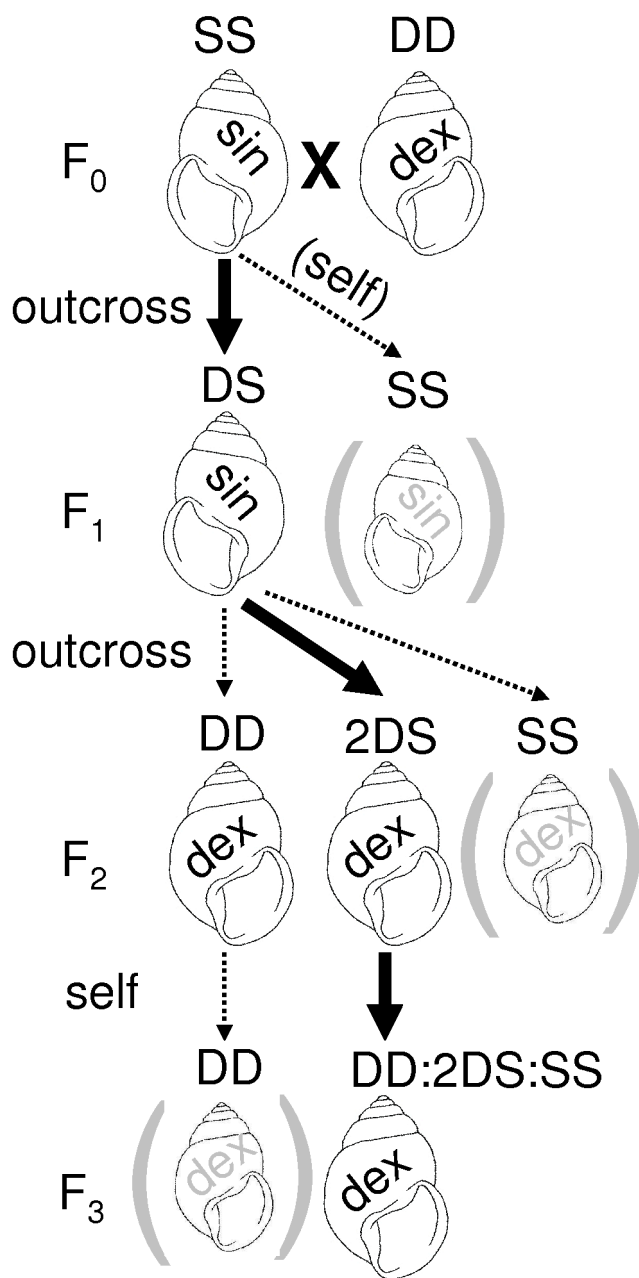


Fig. 2

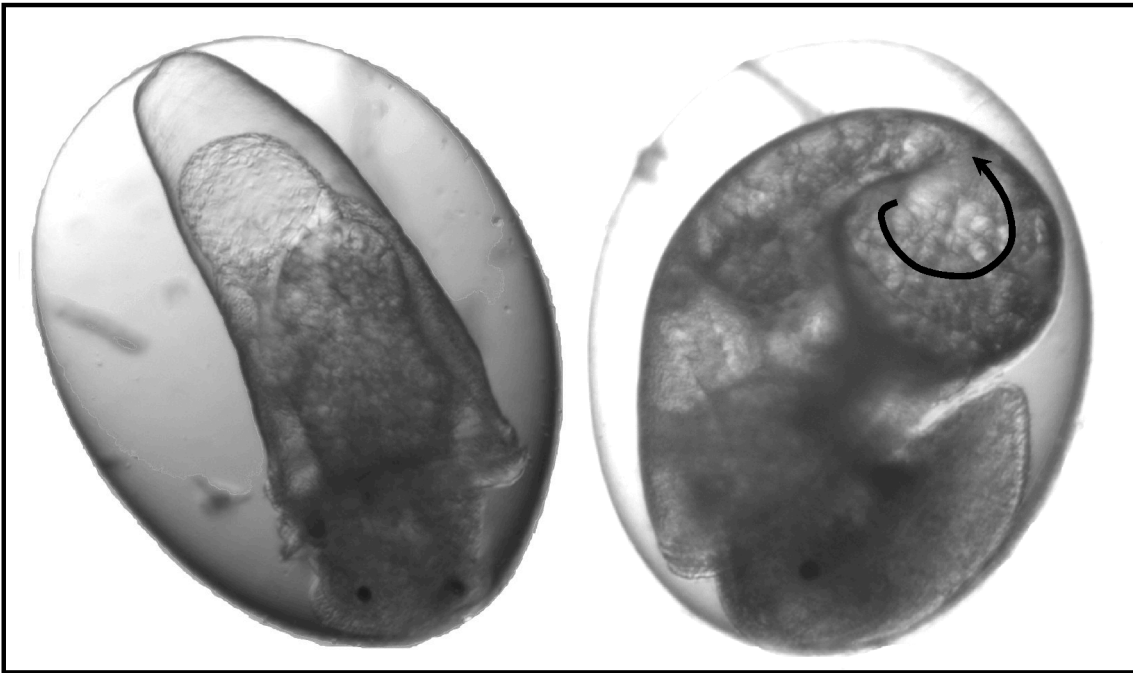


Fig. 3

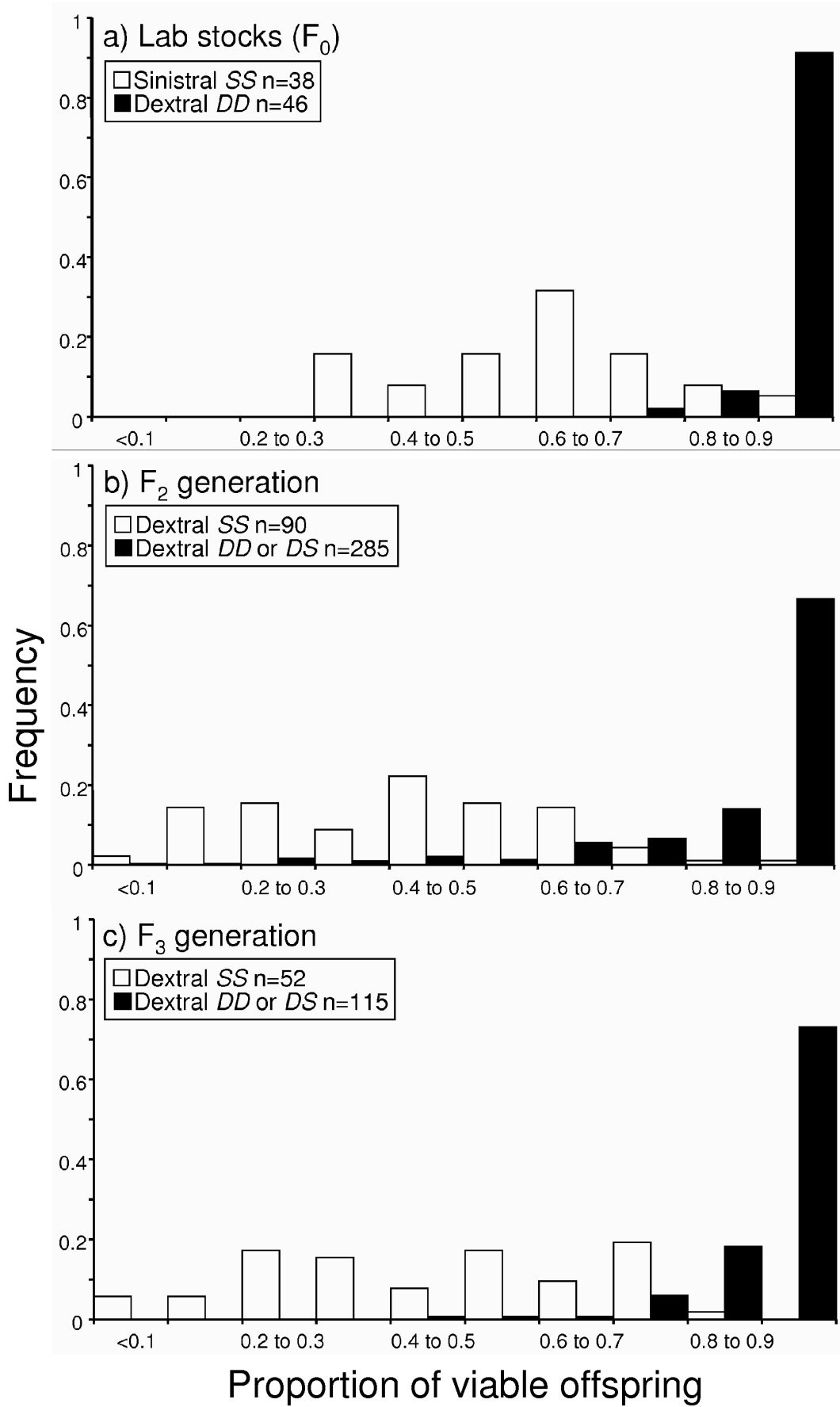


Fig. 4

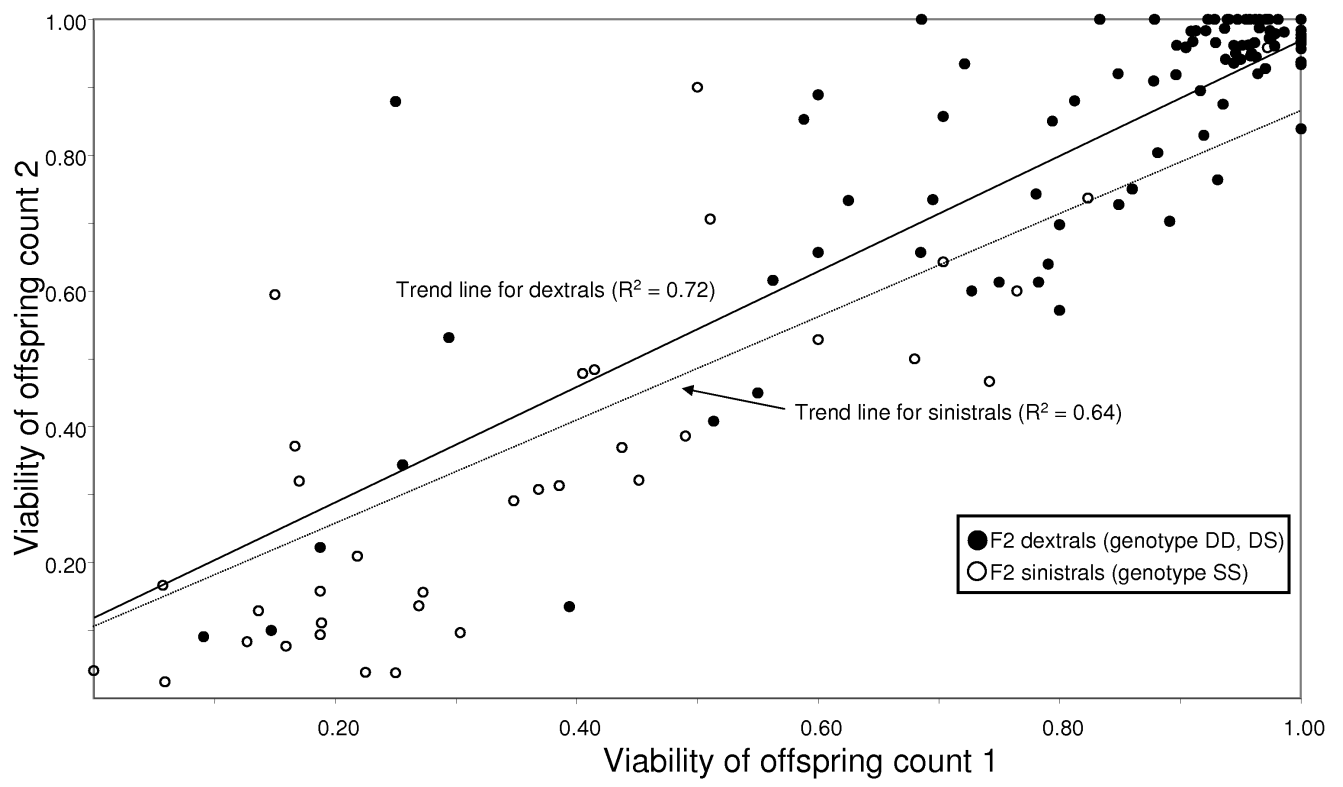


Fig. 5

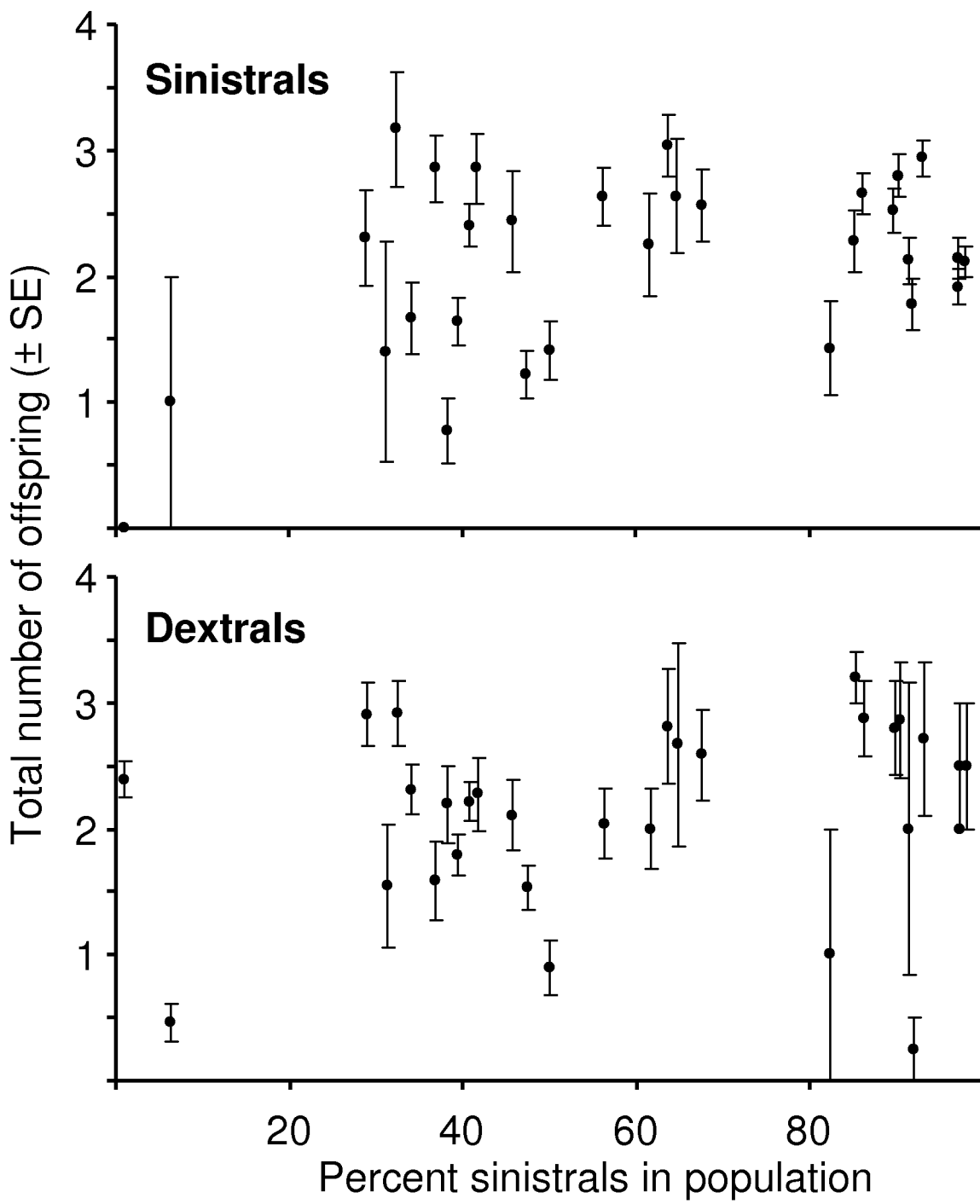
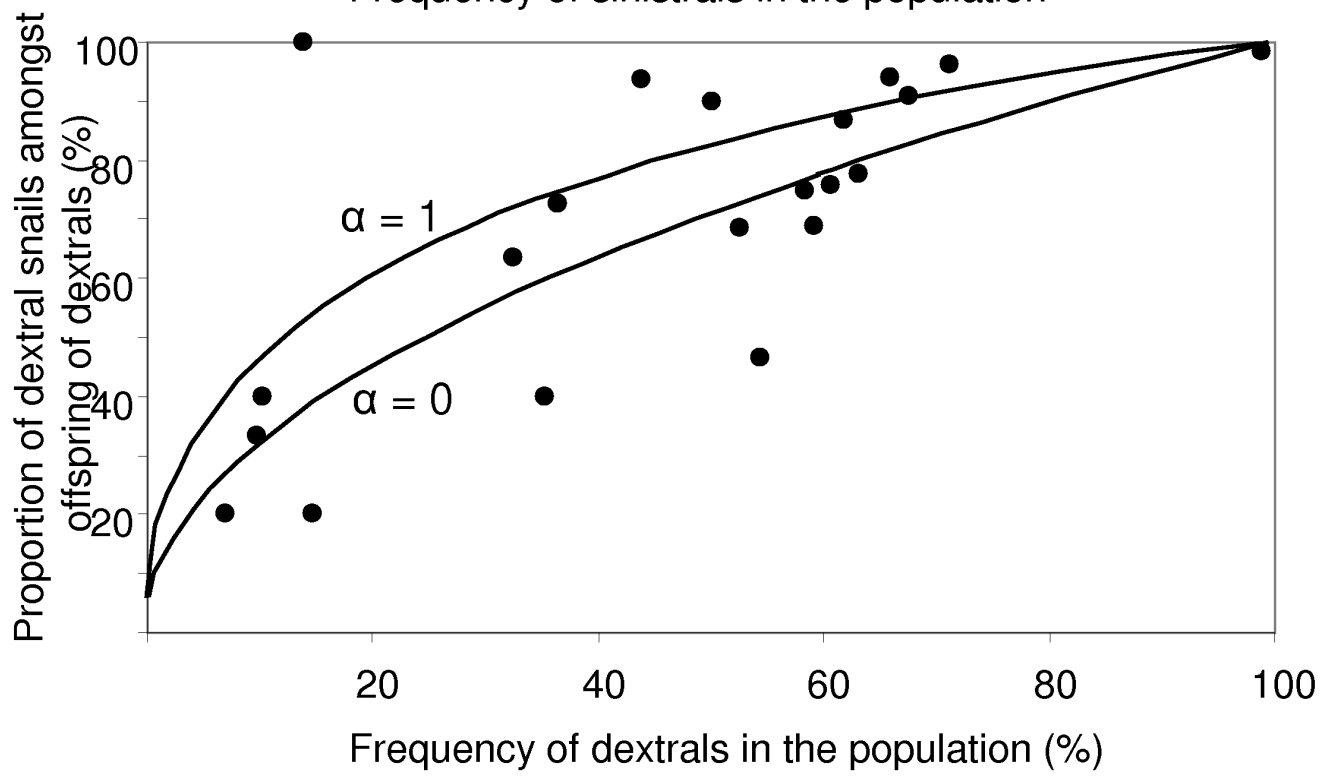
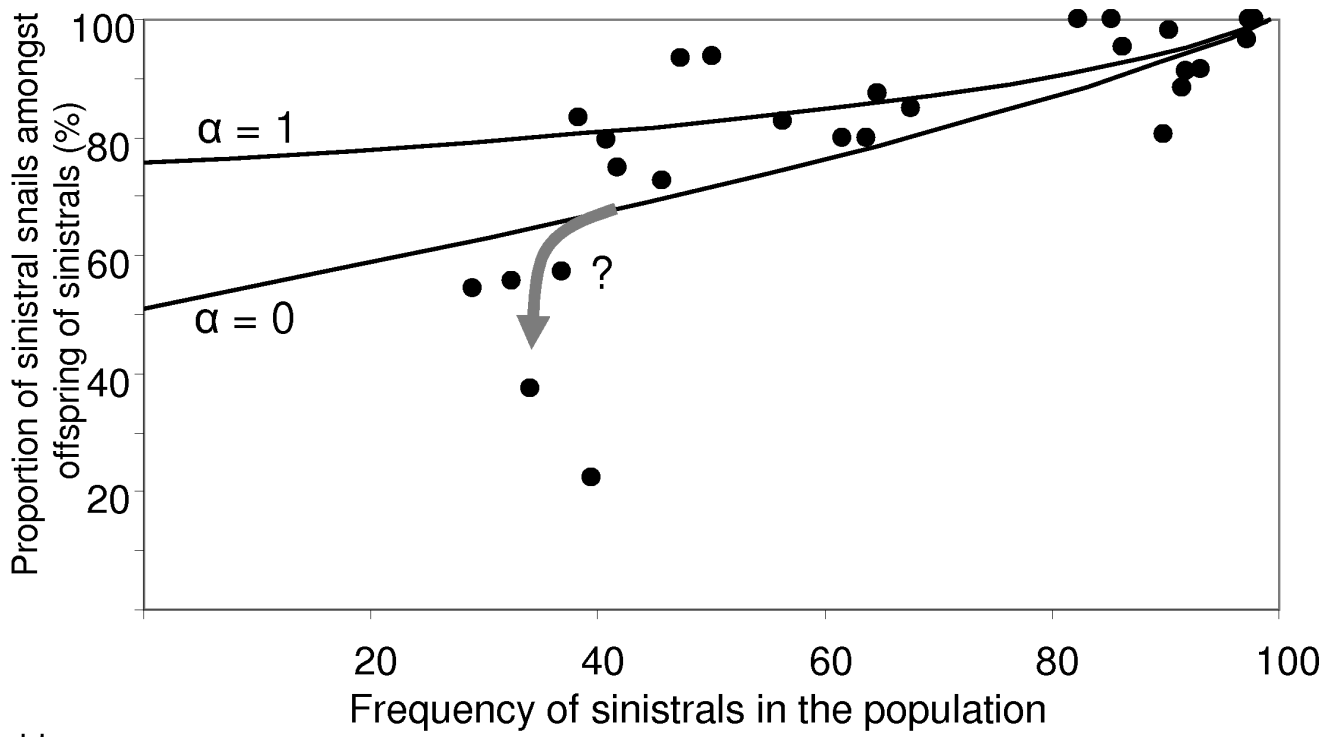


Fig. 6



Supplementary information

The equilibrium, in the absence of sexual selection ($\beta, \Delta=0$) is given by Eqs. 4 in S1:

$$\begin{aligned} su &= 2P - s \\ \alpha w^2 &= sd(d - Q^2) \\ Pd + 2(1 - Q^2 - s) &= w \left(2 + \frac{\alpha}{s} (2P - s) \right) \end{aligned} \quad (1)$$

The proportion of sinistral offspring from sinistral mothers is

$$x = \frac{2sP + 2w = su}{s} \quad (2)$$

Similarly, the proportion of dextrals from dextral mothers is:

$$1 - 2P + 2\frac{w}{d} \quad (3)$$

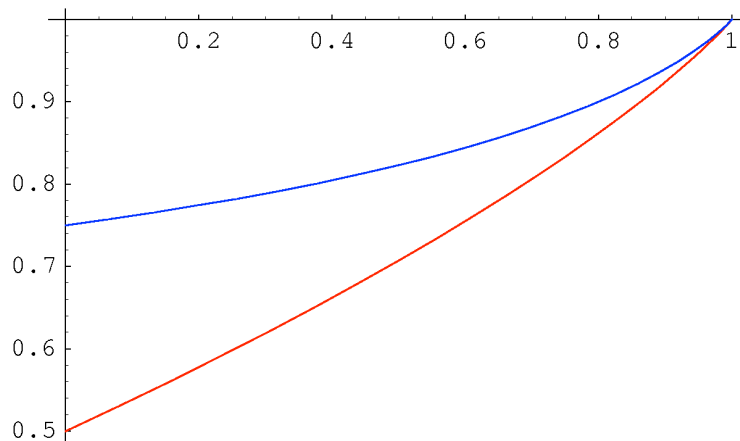
This can be seen from the last row of SI4.

This gives $\{s, su, w\}$ as a function of α, P assuming no sexual selection ($\beta, \Delta=0$).

```
findSoln[alpha_, P_] := Module[{su, w, s, d, Q},
  Select[{s, su, w} /. Solve[{su == 2P - s, alpha w^2 == s d (d - Q^2),
    Pd + 2(1 - Q^2 - s) == w (2 + alpha/s (2P - s))} /. {Q -> 1 - P, d -> 1 - s},
    {su, w, s}], (Im[#] == {0, 0, 0} && Min[#] >= 0) &];
```

This is Fig 6 - proportion of sinistral offspring from sinistrals, plotted against proportion sinistral in the population.

```
ParametricPlot[
  {{ss = findSoln[0, p][[1]]; {ss[[1]], (2 ss[[1]] p + 2 ss[[3]] - ss[[2]]) / ss[[1]]},
  {ss = findSoln[1, p][[1]]; {ss[[1]], (2 ss[[1]] p + 2 ss[[3]] - ss[[2]]) / ss[[1]]}},
  {p, 0, 1}, PlotStyle -> {Red, Blue}];
```



This is the proportion of dextrals amongst offspring of dextral mothers, for $\alpha=0$ (red) and $\alpha=1$ (blue), plotted against the proportion of dextrals in the population.

```

ParametricPlot[
  {
    {ss = findSoln[0, p][[1]]; {1 - ss[[1]], 1 - 2 p +  $\frac{2 \text{ss}[[3]]}{1 - \text{ss}[[1]]}$ }},
    {ss = findSoln[1, p][[1]]; {1 - ss[[1]], 1 - 2 p +  $\frac{2 \text{ss}[[3]]}{1 - \text{ss}[[1]]}$ }},
  },
  {p, 0, 1}, PlotStyle -> {Red, Blue}];

```

