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Experiments to test whether different forms of the land snail *Arianta arbustorum* (LINNAEUS, 1758) (Pulmonata: Helicidae) are reproductively isolated

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(With 3 Tables)

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Zusammenfassung

Eine Versuchsaufstellung wird vorgestellt, die das Vorhandensein von prä- und postkopulativer Isolation zwischen verschiedenen Formen von *Arianta arbustorum* (LINNAEUS, 1758) überprüfen läßt. Präkopulative Isolation kann mit ausgewogenen Partnerwahl-Tests überprüft werden. In diesen Tests werden je zwei Individuen von beiden Formen in eine Beobachtungskammer gesetzt und das Balzverhalten und die Paarbildung protokolliert. Postkopulative Isolation wird vorzugsweise mit Zuchtexperimenten getestet. Der reproduktive Erfolg (Anzahl Eier und geschlüpfte Jungtiere) von homotypischen Paaren (bestehend aus zwei Tiere von der gleichen Population) wird mit demjenigen von heterotypischen Paaren (bestehend aus je einem Tier der verschiedenen Formen) verglichen. Als Beispiel werden die Ergebnisse einer Studie präsentiert, die reproduktive Isolationen zwischen isolierten Populationen von *A. arbustorum* in Schweden und der Schweiz untersuchte.

Summary

An experimental design is presented which tests whether pre- and/or postmating reproductive isolation occurs among different forms of the land snail *Arianta arbustorum* (LINNAEUS, 1758). Premating isolation can be tested in balanced mate-choice tests where the courtship behaviour and the frequencies of homotypic (i.e. within population) vs. heterotypic (between populations) pair formation are examined. Postmating isolation, on the other hand, is preferentially evaluated in breeding experiments, by comparing the reproductive output of heterotypic pairs with that of homotypic crosses of both forms. As an example, the results of a study which investigated reproductive isolation between Swedish and Swiss populations of *A. arbustorum* are given.

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Introduction

The evolution of new species is equivalent to the evolution of barriers to gene flow between populations (MAYR 1963; FUTUYMA 1986). Most species concepts define a species as a group of individuals that are potentially able to interbreed and thereby produce fertile offspring (OTTE & ENDLER 1989). The biological species concept subdivides the mechanisms responsible for the development of barriers into premating and postmating isolation mechanisms (MAYR 1963). Premating mechanisms include behavioural differences in the courtship display (ethological isolation), no transfer of sperm during copulation (mechanical isolation), and seasonal or habitat isolation of individuals (MAYR 1963). Postmating mechanisms, on the other hand, reduce the reproductive success of interspecific crosses. These mechanisms are: (a) gametic mortality (sperm transfer takes place but the eggs are not fertilized), (b) zygote mortality (eggs are fertilized but the zygotes die), (c) hybrid inviability (F_1 hybrids have reduced viability), and (d) hybrid sterility (F_1 hybrids are partially or completely sterile) (MAYR 1963).

Barriers to gene flow (isolating mechanisms) usually arise as by-products of genetic changes in other traits rather than as mechanisms to prevent hybridization (FUTUYMA 1986). Nevertheless, if we adopt the species definition presented above, pre- and/or postreproductive isolation has to occur before we can distinguish individuals of two populations as belonging to separate species. Furthermore, the isolation mechanisms are useful tools in the study of speciation processes. Premating isolation can be studied by carefully observing behavioural differences during controlled mate-choice tests, whereas postmating isolation is preferentially studied in appropriately designed breeding experiments in the laboratory.

In the Gesäuse Mountains (Styria, Austria), the land snail *Arianta arbustorum* (LINNAEUS, 1758) shows a conspicuous variability in shell morphology within a limited area (KOTHBAUER, NEMESCHKAL, SATTMANN & WAWRA 1991) and several subspecies as well as a few sibling species have been described (KLEMM 1973). However, the existence of some of these forms has been questioned because of observed inconsistency in morphological traits among populations (KOTHBAUER, NEMESCHKAL, SATTMANN & WAWRA 1991). The examination of pre- and/or postmating reproductive isolation between different forms of *A. arbustorum* could undoubtedly provide valuable information on the systematic of this species (or species complex). The purpose of my contribution is to describe experiments designed to examine pre- and postmating isolation between different forms of *A. arbustorum*.

In the present context, different populations can refer to different morphs or forms of *A. arbustorum* or even to different subspecies or sibling species. For simplicity, I hereafter refer to different populations.

Premating isolation

Mate-choice tests can be conducted to examine whether premating reproductive isolation occurs between different sympatric (in space and time) populations of *A. arbustorum*. In the following I describe the experimental procedure which could be used to evaluate premating reproductive isolation between two different populations (i.e. forms or subspecies) of *A. arbustorum*. For this purpose, individuals with mating experience from the field (adults with a well developed lip at the shell aperture) will be collected from two populations of distinct morphs of *A. arbustorum*. The snails are preferentially collected in autumn and kept isolated in hibernation until the following spring. In this way the animals are acclimatized to the same conditions, and, most importantly, they have not already mated at the start of the experiment (the peak period of the mating activity of *A. arbustorum* is in spring shortly after arousal from hibernation). The sampling area should not exceed 20 × 20 m to ensure that all individuals belong to the same genetic population (i.e. the same panmictic unit or deme). To facilitate later observations, the adult snails will be marked individually, for example by writing numbers on their shells with a waterproof felt-tipped pen on a spot of correction fluid (Tipp-Ex).

The snails are kept individually in containers of suitable size (at least 200 cm^3). Lettuce *ad libitum* will be provided as food. The bottom of the containers is covered with moist soil mixed with powdered lime. During winter the snails are allowed to hibernate isolated on moist soil in their containers at 2–4°C and in darkness for 5–6 months (no food will be provided). This period corresponds approximately to the length of the hibernation period in the field.

In the following spring mate-choice tests are performed to examine whether premating isolation occurs between the two morphs of *A. arbustorum*. To offer a balanced choice, four snails (two from each population) are placed in a test arena (some kind of transparent plastic container measuring approximately 20 × 15 × 10 cm). The bottom of the container is covered either with moist soil or moist paper towels to maintain snail activity.

Minor behavioural differences in courtship can be the first step towards an incipient prereproductive isolation. To examine any possible behavioural differences, the snails' courtship behaviour will be monitored using a stop-watch. Alternatively, video recording can be used. Records can be taken of the number and duration of homo- and heterotypic meetings, time until initiation of courtship (courtship latency), duration of courtship (time interval from courtship initiation to copulation), and duration of copulation. Observation sessions are terminated when the first copulation occurs in a test arena (since there is no further mate choice for the remaining snails), or after 8 h if no snail initiates courtship behaviour. Individuals that do not initiate courtship during an observation session can be tested again 10–14 days later. As a measure for mating propensity the percentage of snails that mate can be used (i.e. (number of snails that mated) / (total number of snails tested) × 100). For a statistical analysis, at least 24 copulations

are required. According to my experience, 35–40 trials have to be conducted including 70–80 adult *A. arbustorum* from each population.

Assuming random mating between the individuals of both populations, the probability for heterotypic pair formation is ($^1/_2 \times {}^2/_3$) + ($^1/_2 \times {}^2/_3$) = $^2/_3$ and 2 × ($^1/_2 \times {}^1/_3$) = $^1/_3$ for homotypic pair formation ($^1/_6$ for each population). A second kind of expected frequencies of pair formation which takes into account possible differences in mating propensity should also be calculated (an example of this calculation is given in the Appendix). This is done to examine whether any mate preference is the result of a real mate choice or merely a consequence of differences in mating propensities between populations (for further explanation see Discussion). Deviations from random mating are evaluated using χ^2-tests (SOKAL & ROHLF 1981). Successful matings can further be analysed to examine whether particular elements of behaviour influence the snails' choice. In these analyses, the courtship behaviour of individuals involved in heterotypic matings is compared with that of snails in homotypic matings.

Postmating isolation

To test whether postmating reproductive isolation occurs between two populations of *A. arbustorum*, a breeding experiment has to be performed. The animals should be collected as juveniles in the field to avoid prior mating experience and thus interference through stored sperm. The juveniles are kept isolated as described in the previous section until they become sexually mature as indicated by the formation of a lip at the shell aperture. Subsequently, the virgin adults are crossed as follows: population 1 × population 1 (homotypic crosses of population 1), population 2 × population 2 (homotypic crosses of population 2), and population 1 × population 2 (heterotypic crosses). Virgin snails are randomly assigned to a mating partner from the appropriate source population. Pairs are kept in plastic containers (approximate size 15 × 10 × 5 cm) whose bottoms are covered with moist soil mixed with powdered lime to encourage egg-laying. Food (e.g. lettuce) will be provided *ad libitum*. Twelve – 20 replicates of all combinations should be set up. The initial number of replicates should not be too small since one has to expect that some snails will die in the course of the study.

To evaluate the reproductive success of the snails, the containers will be checked weekly for eggs. The eggs are then counted and placed in plastic dishes lined with either moist soil or paper towelling to avoid desiccation of the eggs; each batch of eggs will be kept in a separate plastic dish. The eggs should be incubated at 17–19°C, since the hatching success of *A. arbustorum* eggs decreases when the temperatures exceeds 20°C (BAUR & BAUR 1993). Newly hatched snails ought to be separated daily from remaining unhatched eggs to prevent egg cannibalism and thus a reduction in hatching success (cf. BAUR & BAUR 1986).

The breeding experiment should be run at least for one reproductive season. However, since the snails will not reach sexual maturity simultaneously, two reproductive seasons are required. The reproductive data of homotypic and

heterotypic crosses are compared using either t-test or MANN-WHITNEY U-test (SOKAL & ROHLF 1981).

An example of a study testing premating and postmating isolation

In the following section, I present the results of experiments designed to investigate premating and postmating reproductive isolation in *A. arbustorum* from two geographically isolated populations in Sweden and three populations in Switzerland.

Mate-choice tests

To examine any possible behavioural differences in courtship behaviour and mate preference among snails from different populations and thus to reveal an incipient premating isolation, mate-choice tests were conducted. This study was performed as described above in the section premating isolation, with the following exceptions. Adult individuals of *A. arbustorum* from five populations were collected from two sites in Sweden (referred to as S_1 and S_2) and from three sites in the Swiss Alps (CH_1–CH_3).

S_1: Uppsala, central Sweden (59° 51' N, 17° 40' E). Clearing with rough herbage (mainly *Cirsium arvense*) in a pine-dominated forest.

S_2: Landvetter, near Gothenburg, south-western Sweden (57° 41' N, 12° 13' E). Embankment of a road, covered by rough herbage (*Dactylis* sp., *C. arvense*). 400 km south-west of S_1.

CH_1: Gurnigel, near Berne, Switzerland (46° 44' N, 7° 27' E). Overgrown clearing with rough herbage in a pine forest (1430 m a.s.l.). Approximately 1600 km south-west of S_1.

CH_2: Gantrisch, near Berne, Switzerland (46° 42' N, 7° 27' E). Alpine pasture at 1810 m a.s.l., 4 km south of CH_1.

CH_3: Strela, near Davos, Switzerland (46° 49' N, 9° 48' E). Alpine pasture with scattered scree at 2100 m a.s.l., 180 km east of CH_1 and CH_2.

The following mate-choice tests were conducted: $S_1 \times S_2$, $S_1 \times CH_1$, and $CH_2 \times CH_3$. Three snails (instead of two as suggested above) from each of two populations were tested in the test arenas. This gives a probability for heterotypic pair formation, presupposed random mating among individuals, of $(1/2 \times 3/5) + (1/2 \times 3/5) = 3/5$ and that for homotypic pair formation of $2 \times (1/2 \times 2/5) = 2/5$.

Snail movements in the test arena were seemingly random. When two individuals met, mutual tactile contacts with tentacles occurred. Either the snails separated within 5.5 ± 0.6 min (mean ± SE, range 1–15 min, N = 55), or courtship commenced with oral contacts. Homotypic and heterotypic meetings occurred in frequencies expected by chance (χ^2-test based on total numbers, in all test types $P > 0.1$). The number of potential mating partners (x) encountered prior to courtship initiation with the eventual mate ranged from 0 to 11 (median 3; including repeated counts); its frequency distribution fitted a function with exponential decay

Table 1: Results of mate-choice experiments with *A. arbustorum* from five distant populations.

Test	No.of matings	Mating pairs	Observed no. of matings	Expected for random mating[a])	χ^{2b}	P	Expected matings with different mating propensity[c])	χ^{2b}	P
$S_1 \times S_2$	13	$S_1 \times S_1$	1	2.6			0.5		
		$S_1 \times S_2$	4	7.8	14.05	< 0.001	5.6	1.13	N.S.
		$S_2 \times S_2$	8	2.6			6.9		
$S_1 \times CH_1$	22	$S_1 \times S_1$	7	4.4			2.6		
		$S_1 \times CH_1$	3	13.2	22.55	<0.001	12.6	18.53	<0.001
		$CH_1 \times CH_1$	12	4.4			6.9		
$CH_2 \times CH_3$	14	$CH_2 \times CH_2$	1	2.8			0.9		
		$CH_2 \times CH_3$	6	8.4	8.14	< 0.01	7.0	0.26	N.S.
		$CH_3 \times CH_3$	7	2.8			6.2		

[a]) Expected frequencies do not take into account differences in mating propensity
[b]) d.f. = 1 in all cases
[c]) Obtained by weighing the expected frequencies for random pair formation with the mating propensities (see Appendix)

($y = 27.15\ e^{-0.305x}$, $r^2 = 0.916$, d.f. = 10, $P < 0.001$). Twenty out of the 98 snails (20.4%) which were engaged in copulations in the tests mated with the first partner encountered (13 individuals (18.1%) with a homotypic partner and 7 (26.9%) with a heterotypic partner). Final mate choice (whether homotypic or heterotypic) was not influenced by the number of snails encountered prior to mating (MANN-WHITNEY U-test, $P > 0.1$). Furthermore, the number of homotypic and heterotypic encounters was independent of snail size (SPEARMAN correlation; in all populations, $P > 0.2$).

Courtship did not always lead to copulation. Twenty-six out of the 98 snails (26.5%) had courted unsuccessfully another potential partner before they initiated courtship with their eventual mate. Courtship was more frequently broken off in heterotypic pairs (in 10 out of 23 cases; 43.5%) than in homotypic pairs (in 3 out of 39 cases; 7.7%) (χ^2=11.18, d.f. = 1, $P < 0.001$).

Pair formation (of copulating snails) deviated significantly from random mating (Table 1). In all tests, fewer heterotypic matings occurred than expected under random mating, indicating partial premating isolation. In the $S_1 \times S_2$ and $CH_2 \times CH_3$ tests, however, there were also fewer homotypic matings involving S_1 and CH_2 individuals than expected under random mating (Table 1). Deviations from random mating can partly be explained by between-population differences in mating propensity (percentage of individuals mating). Individuals from populations S_1 and S_2 as well as CH_2 and CH_3 differed significantly in mating propensity ($S_1 \times S_2$ tests: 10.0% vs. 33.3%, χ^2-test, $P < 0.01$; $CH_2 \times CH_3$ tests: 11.6% vs. 29.0%, χ^2-test, $P < 0.02$). As a result, more homotypic matings occurred in the populations S_2 ($S_1 \times S_2$ tests) and CH_3 ($CH_2 \times CH_3$ tests). Individuals from the populations S_1 and CH_1 did not differ in mating propensity (18.8% vs. 30.0%, χ^2-test, $P = 0.08$). Nevertheless, homotypic matings also occurred in excess in

Table 2: Courtship latency and courtship duration in mate-choice experiments with *A. arbustorum*. Values are means (1 SE).

Mating pairs	N	Courtship latency (min)	Courtship duration (min)
$S_1 \times S_1$	1	27.0 –	184.2 –
$S_1 \times S_2$	4	221.2 (10.1)	273.0 (43.0)
$S_2 \times S_2$	8	229.9 (59.2)	257.8 (52.0)
$S_1 \times S_1$	7	198.8 (46.4)	309.9 (66.4)
$S_1 \times CH_1$	3	81.6 (41.0)	241.6 (39.1)
$CH_1 \times CH_1$	12	151.2 (32.6)	308.7 (34.1)
$CH_2 \times CH_2$	1	211.8 –	120.0 –
$CH_2 \times CH_3$	6	84.7 (17.8)	197.6 (33.1)
$CH_3 \times CH_3$	7	181.1 (45.6)	217.8 (26.6)

these populations, even after differences in mating propensity were controlled for, indicating premating reproductive isolation (Table 1).

Courtship latency did not differ significantly between homotypic and heterotypic matings (Table 2; MANN-WHITNEY U-test, in all comparisons $P > 0.1$). Furthermore, courtship duration did not differ between heterotypic and homotypic matings (Table 2; MANN-WHITNEY U-test, in all comparisons $P > 0.4$).

Breeding experiment

To examine whether snail pairs from isolated populations (heterotypic pairs) have a reduced fecundity compared to homotypic pairs and thus to reveal any incipient postmating isolation, a breeding experiment was conducted. This study was performed as described above in the section postmating isolation, with the following exceptions. Snails from the four populations S_1, CH_1, CH_2 and CH_3 were used. Thirty-six individuals from each source population were crossed as follows: 12 $S_1 \times S_1$, 12 $S_1 \times CH_1$, 12 $CH_1 \times CH_1$, and 12 $CH_2 \times CH_2$, 12 $CH_2 \times CH_3$, 12 $CH_3 \times CH_3$. Although breeding data were collected during two reproductive seasons, only those of the second season were used in the analyses. In the first year, snails from different populations varied in time to reach maturity.

In the $S_1 \times CH_1$ breeding, heterotypic and homotypic crosses differed neither in the number of clutches produced, nor in clutch size and hatching success (number of clutches and clutch size: t-test, in all cases, $P > 0.20$; hatching success: MANN-WHITNEY U-test, in both cases, $P > 0.15$). In the $CH_2 \times CH_3$ breeding, heterotypic crosses produced fewer and smaller clutches with a lower hatching success than did homotypic crosses from population CH_2 (number of clutches and clutch size: t-test, in both cases, $P < 0.05$; hatching success: MANN-WHITNEY U-test, $P < 0.01$). In neither of these traits heterotypic crosses differed from homotypic crosses of population CH_3 (the same tests as mentioned above, in all cases $P > 0.08$).

Table 3 shows the number of hatchlings that emerged from homo- and heterotypic crosses. In the $S_1 \times CH_1$ breeding, heterotypic pairs produced as many

Table 3: Number of hatchlings in homo- and heterotypic crosses of *A. arbustorum*.

Cross type	No. of pairs	No. of hatchlings per pair	No. of pairs with successful matings	No. of hatchlings per successful pair
$S_1 \times S_1$	10	63.6 (13.5)	9	70.7 (12.9)
		N.S.		N.S.
$S_1 \times CH_1$	9	87.5 (28.8)	8	98.4 (30.2)
		N.S.		N.S.
$CH_1 \times CH_1$	8	91.4 (16.7)	8	91.4 (16.7)
$CH_2 \times CH_2$	9	189.3 (20.2)	9	189.3 (20.2)
		**		**
$CH_2 \times CH_3$	10	69.1[a] (23.1)	8	86.4 [b] (25.4)
		N.S.		N.S.
$CH_3 \times CH_3$	9	71.3 (14.0)	8	80.3 (12.2)

Data are means (1 SE). P – values for intercross comparisons result from t – tests.
** $P < 0.01$, N.S. not significant
a) The number of hatchlings in heterotypic crosses differs significantly from the mean value of both homotypic crosses (130.3 hatchlings; $t = 2.649$, d.f. = 9, $P < 0.05$).
b) The number of hatchlings in heterotypic crosses does not differ from the mean value of both homotypic crosses (134.8 hatchlings; $t = 1.906$, d.f. = 7, $P = 0.09$).

offspring as did homotypic crosses. This indicates that individuals of both populations separated by 1600 km have maintained reproductive compatibility. In the $CH_2 \times CH_3$ breeding, fewer hatchlings derived from heterotypic than from homotypic crosses of the population CH_2, suggesting a reduced compatibility. The number of hatchlings deriving from heterotypic crosses differed from the average number of young of both homotypic crosses, when all pairs were considered (Table 3). However, when pairs that failed to reproduce were excluded from the analysis, the number of hatchlings produced by heterotypic crosses did not differ from the average number of young of both homotypic crosses (Table 3). Considering all crosses, five of the 55 pairs failed to produce any offspring at all: two homotypic pairs (5.6%) and three heterotypic pairs (15.8%) (Table 3).

The number of hatchlings produced varied greatly within each group of matings (i.e. $S_1 \times S_1$, $S_1 \times CH_1$ and so on); even when pairs that failed to produce any offspring were excluded, the number of offspring per pair showed two- to twenty-fold differences within each group. In both breeding experiments, the group of heterotypic pairs showed a variation twice as high as those in the corresponding group of homotypic pairs. Due to the great within-group variation, it is difficult to assess the real extent of differences in the number of offspring produced between the groups.

Discussion

Human beings often tend to classify specimens into discrete categories although the interindividual variability can be substantial. MAYR (1963) calls this phenomenon "typological thinking". According to FUTUYMA (1986) the

classification of most of our taxonomic categories is quite arbitrary. Some authors have argued that the subspecies concept is so arbitrary that it should be abandoned (WILSON & BROWN 1953). Even the biological species concept ("species are groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups" (MAYR 1942)), which is the most widely accepted among species concepts, is sometimes difficult to apply. The most severe criticism of the biological species concept is that it cannot be applied to selfing and asexually reproducing organisms. However, for sexually reproducing organisms the biological species concept is testable.

The purpose of this contribution was to present an experimental procedure to examine reproductive isolation in (usually outcrossing) land snails from different populations. The procedure described above can be discussed on several points. For example, the importance of collecting snails within a limited area of 20 × 20 m can be questioned. This advice has a profound theoretical background. It is based on the definition of a genetic population in a continuously distributed species. In such a case, the effective population size (i.e. the size of an idealized population equivalent genetically to a real one) depends on the mode of reproduction, the population density and the snails' dispersal capacity (cf. WRIGHT 1978). Since land snails have a limited dispersal capacity, the area covered by a genetic population (i.e. the neighbourhood area) is rather small.

In the example presented, six snails (three from each of two populations) were used in the mate-choice tests. However, for future studies I would recommend to use four snails (two from each population) in each test. This allows more replicates to be performed with the same total number of snails.

Some students of evolution claim that differences in mating propensity are the first step towards reproductive isolation and thus compare observed and expected frequencies of pair formation to test reproductive isolation (e.g. MARKOW 1981, MEFFERT & BRYANT 1991). Others maintain the more conservative view that behavioural data have to be corrected for differences in mating propensity among populations before observed and expected frequencies of pair formation can be compared (J. J. D. GREENWOOD, personal communication; see Appendix).

In the breeding experiment, snails can be kept constantly in pairs as described in the example. As an alternative, individuals can be maintained singly, but allowed to mate with a particular partner at regular intervals. The advantage of the second approach is that the reproductive performance of individuals and not that of pairs can be examined. In the case of asymmetry in the effect of reproductive isolation between populations, this advantage might be substantial. This approach, however, is more work consuming. To prolong the breeding experiment for a second reproductive season can appear to be a waste of time. However, this step might be necessary in most cases for two reasons. First, the variation in time required to reach maturity after hibernation is great and thus the length of the first reproductive season extremely variable for this species. Second, two newly-adult snails sometimes fail to mate with each other in their first attempt, most probably because they lack prior mating experience (BAUR & BAUR, in preparation). In the

field, most virgin snails may meet partners with mating experience and therefore matings between virgins may be relatively rare. However, according to my experience, two virgin snails will successfully mate in their second or third attempt.

The results of the example presented show that reproductive isolation must not depend on the geographical distance between the populations considered. A Swedish and a Swiss population of *A. arbustorum* showed partial premating isolation but simultaneously a high degree of reproductive compatibility. On the other hand, two Swiss populations showed a partly reduced reproductive compatibility. Thus, closely-situated populations are as likely as more remote ones to show reproductive isolation.

To summarize, mate-choice tests and breeding experiments are useful tools to test pre- and postreproductive isolation among populations of the land snail *A. arbustorum*. These procedures may provide basic information to confirm or reject the present systematics of this species.

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Appendix: Calculation of expected frequencies of pair formation which take into account differences in mating propensity. As an example the observed pair formations in the $S_1 \times S_2$ mate-choice experiment were used.

Observed mating propensity:
$S_1 = 6/26 = 0.23$
$S_2 = 20/26 = 0.77$
Probability that a S_1- or S_2-snail will copulate presupposed that a S_1-snail is ready to mate (the factor 1.805 makes that the overall probability is equal to 1.0000):
$S_1 = 0.23 * 2/5 * 1.805 = 0.1661$
$S_2 = 0.77 * 3/5 * 1.805 = \underline{0.8339}$
1.0000
Probability that a S_1- or S_2-snail will copulate presupposed that a S_2-snail is ready to mate (the factor 2.242 makes that the overall probability is equal to 1.0000):
$S_1 = 0.23 * 3/5 * 2.242 = 0.3094$
$S_2 = 0.77 * 2/5 * 2.242 = \underline{0.6906}$
1.0000

Expected frequencies of pair formation (corrected for differences in mating propensity):		Expected number of matings:
$S_1 \times S_1$:	$0.23 * 0.1661 = 0.038$	$0.038 * 13 = 0.49$
$S_1 \times S_2$:	$0.23 * 0.8339 = 0.192$	$0.192 * 13 = 2.50$
$S_1 \times S_2$:	$0.77 * 0.3094 = 0.238$	$0.238 * 13 = 3.09$
$S_2 \times S_2$:	$0.77 * 0.6906 = \underline{0.532}$	$0.532 * 13 = \underline{6.92}$
	1.000	13.00