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Analysis of the mating behaviour of *Arianta arbustorum* (Helicidae, Gastropoda): isolation tendencies between *A. a. arbustorum* (Linnaeus, 1758) and *A. a. styriaca* (Frauenfeld, 1868)

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Abstract: The mating behaviour (from courtship to copulation) of two morphologically distinct populations of *Arianta arbustorum* from two geographically distant regions were compared under laboratory conditions. The individuals of the two populations (A, B), representing the two subspecies *Arianta arianta arbustorum* and *Arianta arbustorum styriaca*, significantly differed in shell characters: individuals with globular, not umbillicated shells and individuals with flat and umbillicated shells. One aim of the study was to describe mating behaviour in *A. arbustorum* in detail, including particular behaviour such as biting, love-dart usage, mucus ejection and mucus eating, formation of a head wart as well as eversion of lobes. Moreover, possible pre-copulative isolation between the two populations should be investigated. Mating trials were performed and observed with partners from the same population (A x A, B x B) as well as combining partners from the two populations (A x B). Altogether, 252 pairs (84 pairs per group) were observed. The process of courtship to copulation was divided into five consecutive phases (P1–P4: pre-copulative phases, P5: copulation), each characterized by a certain conduct of behaviour. Phase P3 has evidently a key role in courtship behaviour and was observed in all pairs that successfully copulated. Although in mixed mating pairs consisting of individuals from the two populations (A x B), mating success was generally lower, this was observed also for B x B matings, when considering only individuals without any previous mating experience. The results are not conclusive and experimental bias cannot be excluded. Nevertheless, the results provide hints towards prezygotic isolation tendencies and may stimulate further studies.

Keywords: *Arianta arbustorum* ssp., courtship behaviour, mating behaviour, prezygotic isolation, morphotypes, Helicidae

Zusammenfassung: Das Paarungsverhalten (von Balz bis zur Kopulation) von zwei Populationen von *Arianta arbustorum* aus zwei geografisch entfernten Regionen wurde unter Laborbedingungen analysiert. Die Individuen der beiden Populationen (A, B), die taxonomisch zwei Unterarten zugeordnet werden, unterschieden sich signifikant in Schalenmerkmalen: Individuen mit kugeligen und ungenabelten Schalen (A = *A. a. arbustorum*) und Individuen mit abgeflachten und genabelten Schalen (B = *A. a. styriaca*). Ein Ziel der Untersuchung war die detaillierte Beschreibung des Paarungsverhaltens von *A. arbustorum*. Neben der Beschreibung des Ablaufes der Balz bis zur Kopulation wurden auch spezielle Verhaltensweisen, wie gegenseitiges Beißen, Einsatz von Liebespfeilen und Schleimabsonderungen, Ausbildung von Kopfwarzen und Ausstülpen eines löffelförmigen Organs („Reizkörper“) dokumentiert. Ein weiters Ziel der Studie war, mögliche präkopulative Isolationsmechanismen zwischen den beiden Populationen zu untersuchen. Paarungsversuche wurden daher sowohl mit Partnern aus derselben Population (A x A, B x B) als auch mit kombinierten Partnern aus den beiden Populationen (A x B) durchgeführt und dokumentiert. Insgesamt wurden 252 Paare (84 Paare pro Gruppe) beobachtet.

Der Balz- und Paarungsprozess wurde in fünf aufeinanderfolgende Phasen unterteilt (P1–P4: präkopulative Phasen, P5: Kopulation), die jeweils durch ein bestimmtes Verhalten gekennzeichnet sind. Die Phase P3 nimmt offensichtlich eine Schlüsselstellung im Balzverhalten ein und wurde bei allen Paaren beobachtet, die erfolgreich kopulierten. Obwohl bei Paarungen, die aus Individuen der beiden Populationen (A x B) bestanden, der Kopulationserfolg im Allgemeinen geringer war, wurde dies auch bei B x B-Paarungen beobachtet, wenn nur Individuen ohne vorherige Paarungserfahrung berücksichtigt wurden. Die Ergebnisse sind also nicht schlüssig, und eine experimentelle Verzerrung kann nicht ausgeschlossen werden. Dennoch geben die Ergebnisse Hinweise auf präzygotische Isolationstendenzen und sollten zu weiteren Untersuchungen anregen.

Schlüsselwörter: *Arianta arbustorum* ssp., Balzverhalten, Paarungsverhalten, präzygotische Isolation, Morphotypen, Helicidae

Preamble

The study described in the present paper was performed in the years 1994 to 1995 and presented by Gabriele Baumgartner (1997) in German as a diploma thesis. Since the results were never published in an international journal, they remained rather unnoticed. Considering the fact that comparably detailed observations on land snail behaviour are very rarely found in the literature, we decided to summarize the major outcomes of that work in English to make it available for a wider readership. Here, we concentrate on the description of courtship and mating behaviour. In the discussion we interpret these observations considering also more recent literature.

Introduction

Reproductive isolation mechanisms are of major importance for speciation processes (Mayr 1963, Futuyma 1990) and even subtle differences in mating behaviour may act as or lead to the formation of reproductive barriers (e. g., Adamo & Chase 1988, Reise 1995). Unfortunately, for many species there is no detailed information on mating behaviour available. This is particularly the case for invertebrates as land snail species, representatives of which were investigated in the present study. For many land snail species conspicuous morphological shell characters are well described, nevertheless biological data are often insufficient or missing.

In this case study the mating behaviour of the land snail species *Arianta arbustorum* (Linnaeus, 1758) was investigated in two populations representing different morphotypes. *Arianta arbustorum* is a simultaneously hermaphroditic land snail, widely distributed throughout Europe from the Pyrenees to Ukraine with occurrences on the British Islands, Scandinavia and Iceland (Kerney et al. 1983). The range of the species has been expanding throughout the last decades with populations in Fennoscandia, the Baltic region as well as in northwestern Russia (Bondareva et al. 2020). In Canada it was accidentally introduced and became an invasive species (McAlpine et al. 2009, McAlpine & Forsyth 2014). The species occurs from the lowlands up to 3000 m above sea level (Ehrmann 1933, Kerney et al. 1983, Fechter & Falkner 1989). It shows an enormous variability in shell characters (e.g., Cook & King 1966, Burla & Stahel 1983, Baminger 1997). Thus, many subspecific taxa have been described, yet, partly based on only a few individuals (comp. Ehrmann 1933, Klemm 1974, Frank 1992, Baminger 1997). In general, due to the partially overlapping distribution of various morphotypes, the individual assignment to a subspecies by shell characters has been considered problematic (Mildner 1981, Nemeschkal & Kothbauer 1988, Kothbauer et al. 1991, Baminger 1997). Neither allozyme studies of Austrian *Arianta* populations (Haase & Bisenberger 2003) nor mitochondrial (mt) DNA sequences (cytochrome c oxidase subunit 1 gene; *COI*) (Haase et al. 2003, Haase & Misof 2009, Haase et al. 2013) allowed unambiguous genetic assignment of subspecies, populations or morphotypes. A recently published tree based on mitochondrial *COI* sequences provides an overview of phylogenetic relationships among representatives of *Arianta* ssp. including the subspecies *Arianta arbustorum doriae* (Paulucci, 1878) (Hausdorf & Walther 2021). The patchy pattern of haplotype distribution in the Alpine region was explained by postglacial colonization of formerly glaciated habitats from multiple refugia (Haase et al. 2003, Gittenberger et al. 2004, Haase & Misof 2009, Haase et al. 2013, Hausdorf & Walther 2021). Retrospective niche modelling performed by Bondareva et al. (2020) confirmed previous assumptions of multiple glacial refugia of *A. arbustorum* in Europe.

Two highly distinct subspecies of *A. arbustorum* are of special interest and have been included in the present study: individuals with globular and not umbilicated shell representing the nominate form *A. arbustorum arbustorum* (Fig. 1A) are clearly distinguishable from those with extremely flat and wide open umbilicated shell (Bisenberger 1993, Baminger 1997). The latter form corresponds to *Arianta arbustorum styriaca* (Frauenfeld, 1868) (Figs. 1B, 2 & 5), with a rather restricted distribution in mountainous regions of the Northeastern Calcareous Alps in Styria and Upper Austria and represents an alpine endemic (Reischütz & Reischütz 2008). *Arianta arbustorum styriaca* was assumed to represent a relic form that survived the glacial periods in a Northeastern Alpine refugium (Gittenberger 1991, Baminger 1997). Besides these conspicuous morphotypes (globular vs. flat) intermediate forms are also found and, besides pure populations, there are populations with high variation in shell morphology (Klemm 1974, Gittenberger 1991, Baminger 1997). Adjacent populations were discovered in the Gesäuse mountains (Styria), each of them composed solely of one of the two morphotypes with no intermediate forms. This raised the question whether there might be reproductive barriers between *A. a. styriaca* and the nominate form *A. a. arbustorum.* Information on reproduction barriers between the different morphotypes of *A. arbustorum* could give valuable information on possible gene flow, and thus the intraspecific taxonomy of this species. Prior to the present study no detailed information was available about mating behaviour of *A. arbustorum*, except the duration of courtship, which was reported by several authors to last 2 to 18 hours (Hofmann 1923, Frömming 1954, Baur & Baur 1992).

Two geographically separated populations, which significantly differ in shell characters, were investigated in the present study. The major aim was to gain detailed

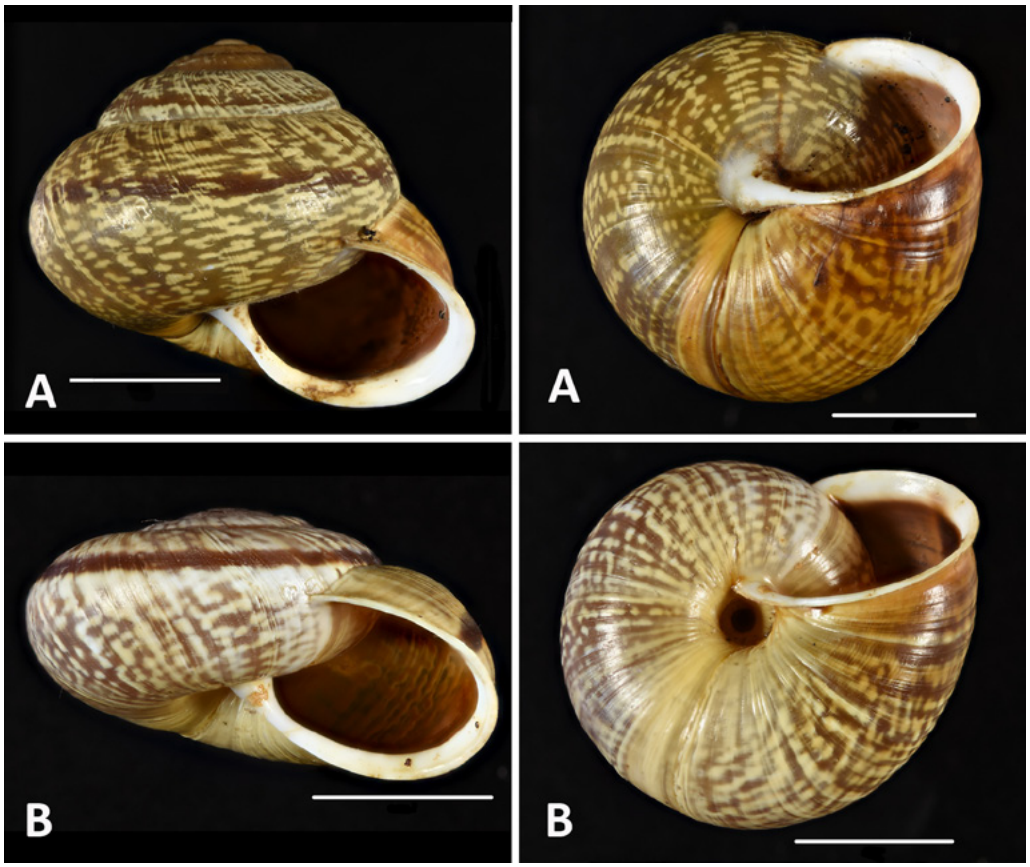

Fig. 1: A: *Arianta a. arbustorum*, Klosterneuburg-Kierling, leg. A. Dimmel, NHMW-MO 75000/E 58645; B: *Arianta a. styriaca*, Gesäuse, Haindlkar, leg. A. Bisenberger, NHMW MO 89820. Scale 1 cm each. Photos: Sara Schnedl, NHMW

descriptions of the mating behaviour of *A. arbustorum* and to assess behavioural differences comparing the two populations. Furthermore, mating behaviour was studied in mixed couples of the two populations to observe differences of mating and copulation frequency in the three mating groups investigated.

Material and Methods

Specimens and populations

Individuals of *A. arbustorum* from two different populations were investigated. The individuals from the lowland population (n = 75) of the subspecies *A. a. arbustorum* were characterized by globular and not umbilicated shells. They hatched in May 1994 from five clutches collected at the River Danube wetlands near Klosterneuburg, Lower Austria (near Vienna, 196 m above sea level). This population is hereafter denoted as "population A". The individuals from the mountain population of the subspecies *A. a. styriaca* (n = 78) were characterized by extremely flat and wide open umbilicated shells. Juveniles were collected in September 1994 at the Haindlkar, Gesäuse, Styria (1350 m above sea level). This population is hereafter denoted "population B". The individuals of both populations originated from an area of 20 × 20 m each to ensure that individuals belonged to one population (Williamson et al. 1977).

Laboratory conditions

Juvenile individuals (maximum number of whorls: 4.5 - counted according to Kerney et al. 1983) of populations A and B were allowed to hibernate for four months (November 1994 to February 1995) in a garden barn at 3 ± 2° C to synchronize and to adapt them to the same conditions. At the end of February 1995, juvenile snails were kept individually in transparent plastic boxes (length × width × height = 11 × 8 × 9 cm) to obtain adults without any mating experience. The bottom of each box was covered with 3 cm moist soil enriched with pulverized chicken-eggshells. Small holes in the top of each box guaranteed ventilation. The boxes were kept in the laboratory at 18 ± 3° C, 60 % air humidity and natural light conditions, and cleaned two to three times per week. Snails were fed with lettuce and carrots (both from ecological farming) ad libitum. In the middle of April 1995, most snails reached sexual maturity as generally indicated by the formation of a lip at the shell aperture (e.g., Lang 1904, Künkel 1916, Boettger 1952).

Adult individuals were marked individually by writing a number on their shells with a black permanent marker on a

Fig. 2: *A. a. styriaca* (BxB) at courtship in a trial box at phase 2 (P2), moving around in a circle. Photo: G. Baumgartner

Table 1. Duration of the phases (P1 – P5) of *A. arbustorum*. All pairs which had copulated (n = 42) are included. Data of mating groups A x A, B x B, and A x B were pooled. Durations are only for illustration.

	Mean duration [min]	95 % confidence interval of the mean
P1	22	9–35
P2	30	21–39
P3	265	228–302
P4	19	4–34
P5	121	16–127

spot of white correction fluid (Tipp-Ex). Shell height and width were measured with a vernier calliper to the nearest 0.1 mm (shells oriented according to Kerney et al. 1983). The degree of umbilication was estimated according to a rating scale (comp. Kothbauer et al. 1991).

Trials

Adult snails (randomly chosen) were placed pairwise in transparent plastic boxes (length × width × height = 14 × 7 × 7 cm). The bottom of the boxes was covered with moist filter paper to facilitate snail activity. In total, on 28 days from the end of April until the end of June 1995 observations of snail pairs were conducted. At each trial, nine pairs were observed as follows: three pairs of group A × A, three pairs of group B × B and three pairs of group A × B ("mixed pairs"). Activities, in particular mating behaviour were protocolled. The beginning of mating behaviour was defined as the first reciprocal tactile tentacle or oral contact. Observation sessions stopped after 17 hours at the latest or after six hours if no snail initiated any mating behaviour. In case of copulation, snails were excluded from further tests. If no copulation happened, the snails were included again in the tests after 10 to 14 days with a different partner. All trials took place in an observation room with open windows (temperature 18 ± 4° C) and under electric light. The snails are usually active at dusk and in the first half of the night (Cameron 1970, Bailey 1975, Baumgartner et al. 2000). Also, when the weather is rainy, snails are active during daytime (Tompa 1984). Thus, the tests were conducted mainly at night-time, but, at rainy and thundery weather, also during daytime, depending on the snails´ activities (Cameron 1970, Bailey 1975, Tompa 1984).

Fig. 3: *A. a. arbustorum* (A x A) copulating (P5) in a trial box. Snails are partially retracted into their shells, the tentacles are gradually retracted, sometimes completely, and the lobes are spoon-shaped and not expanded. Photo: G. Baumgartner

Altogether, 252 pairs (84 pairs per group) were observed. In 72 pairs (24 pairs per mating group: A × A, A × B, B × B) both partners had no mating experience and were tested for the first time. In the remaining 180 pairs at least one partner had been used in one or several tests before.

Statistics

T-tests were applied to examine differences in shell morphology of the two populations (A, B). A Chi-squared test was carried out to assess possible differences in mating behaviour between the three groups A × A, B × B and A × B. For the Chi-squared test, only pairs without mating experience were included (24 pairs per group).

Results

The average of the shell-shape (height-width-ratio) was 0.76 (n = 75) in population A and 0.50 (n = 78) in population B. Individuals from the two populations differed significantly in shell-shape (t-test of independent samples; t = 23.2281, d.f. = 75.5, $p < 0.001$). Additionally, the snails from population A were evidently less umbilicated (Median = 1) than those from B (Median = 7).

The observation of the courtship and mating process of *A. arbustorum* resulted in the description of five consecutive phases: phase 1 to phase 5 (P1–P5; Fig. 4). Each phase is characterized by a certain type of behaviour which may be repeated several times during the same phase and may differ in duration and frequency. The behaviour patterns of the mating partners were identical but not necessarily synchronized. The different phases in general followed each other in a fixed order, but the phases P1, P2, P4 as well as the combinations P1/P4 and P2/P4 were omitted sometimes. Average durations of the phases are compiled in Table 1 indicating that P3 is the longest phase, followed by P5. P3 was always observed when pairs were successful in copulation. Yet, occurrence of P3 did not necessarily mean that a successful copulation followed.

Description of the phases

In Fig. 4 behaviour of snails and their relative positions to each other during the five phases are illustrated.

Phase 1 (P1): Snails make their first contact with tentacles and lips. Contacts are occasionally and short. The genital aperture of both snails is slightly enlarged.

Phase 2 (P2): Snails touch each other repeatedly with tentacles and lips. The genital aperture of both snails is distinctly enlarged. After touching they move their heads on the right side of the partner and try to bring their genital apertures close together. This head-to-head-position lasts until one snail moves away or both snails move away synchronously and subsequently move around in a circle or semi-circle clockwise or counter-clockwise. These movements are the only interruption of contact between the two partners. Within a few minutes they return and approach each other again and start touching each other.

Phase 3 (P3): P3 is similar to P2, with the essential difference that snails evert their lobes, which are spoon-shaped structures of the genital atrium (comp. Hofmann 1923). When the partners are close to each other in head-to-head-position, the lobes are enlarged and loose the spoon-shaped look. When snails start moving in a circle again, the lobes decrease in size and only at the next contact they swell again to regain the spoon-shaped form. Evidently, P3 has a key role in courtship behaviour because it was always observed when pairs copulated. The eversion and enlargement of lobes could be essential for the significance of P3 regarding a successful mating. In the present study, once an everted lobe of a snail was accidently touched (because the trial was stopped due to an inactive partner), when turning this snail back into its box, the penis everted immediately. This was confirmed by testing further snails with already inactive partners by touching the everted lobes intentionally. This initiated in most cases that they everted their penises.

Phase 4 (P4): Snails lay side by side and press their everted spoon-shaped lobes against the mouth of the partner. In this position penial eversion rapidly occurs. If only one

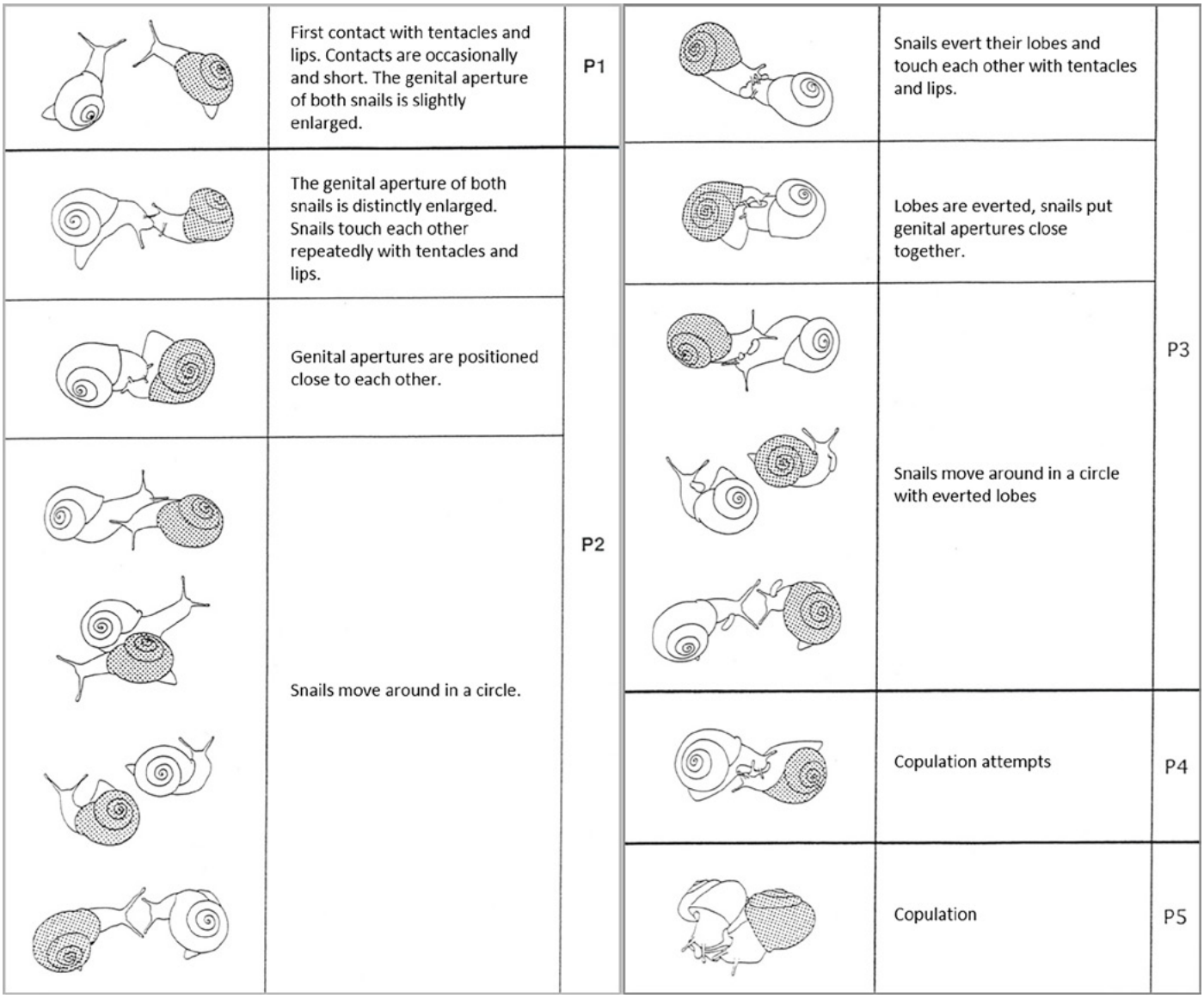

Fig. 4: Courtship and mating process of *A. arbustorum* shown in five consecutive phases (P1–P5). After Baumgartner (1997)

of the partners achieves successful intromission both individuals retract the penis. Such copulation attempts may be repeated several times. When snails lose their optimal position to copulate, they move in a circle again, similar to P2 and P3. At the next contact, snails try to get immediately in head-to-head-position and new attempts to copulate start.

Phase 5 (P5): Simultaneous reciprocal copulation starts, when both snails achieve successful introduction of the penis. The snails are partially retracted into their shells, the tentacles are gradually retracted, sometimes completely, and the lobes are spoon-shaped and not expanded. In this position the snails remain for nearly the whole duration of copulation. At the transfer of the spermatophores, which are formed and filled with sperms during copulation (comp. Hofmann 1923), the snails move their heads laterally which causes the penis to be drawn slightly out of the vagina. Pulsations along the penis can be seen. After the transfer of spermatophores, the penises are pulled out of the vagina of the partner synchronously or asynchronously. The everted genital organs are withdrawn. Briefly after copulation, the end of the spermatophore can be seen as thin, white filament hanging out of the genital aperture. Subsequently, the musculature of the foot soles performs continuous peristaltic waves facilitating the uptake of the complete spermatophore through the genital aperture.

Additional pre-copulative mating patterns

Biting: Some snails were biting the partner during P2 and P3. Considering all 42 pairs that successfully copulated, biting occurred in 17 pairs (out of 22) in group A × A, in 14 pairs (out of 16) in group B × B, and in three pairs (out of four) of group A × B. The bitten animal reacted suddenly by retracting into the shell. After short time they started mating again.

Love-dart: The love-dart of helicids is a calcareous structure, which is sometimes pushed into the body of the copulation partners during mating. Throughout the present investigation (among all mating experiments), only one snail of group A × A displayed dart-shooting in P3. Another love dart was found near the genital aperture of a snail of group A × A after the mating trial. Only one partner of this pair achieved successful intromission for about 50 minutes, but spermatophore transfer did not occur.

Table 2. Mucus ejection and eating of mucus during the mating behaviour of *A. arbustorum*. All pairs which had copulated (n= 42; number for each group in parentheses) were included. Abbreviations: A, individuals from the lowland; B, individuals from the mountain.

	A × A (22)	B × B (16)	A × B (4)
Mucus ejection			
Both individuals	6	2	1
One individual	11	9	3
No mucus ejection	5	5	0
Mucus eating			
Both individuals	3	1	1
One individual	7	7	3
No mucus eating	12	8	0

Fig. 5: *A. a. styriaca,* Gesäuse mountains, mating in the field; phase 3 (P3), head-to-head-position, the lobes are enlarged and not spoon-shaped. Photo: H. Sattmann, NHMW

Mucus ejection and mucus eating (Table 2): During P3 and/or P4 several snails secreted a milky-white, viscous mucus out of the genital apertures. At the end of a short pause, sometimes the partner ate the mucus mass, or the snail ate its own mucus. Table 2 shows the number of individuals ejecting and / or eating mucus in each of the three mating groups.

Head-wart: In the present study, a head-wart, i.e., a swelling between the eye bearing tentacles, was observed in ten individuals during the mating trials – in four pairs of group A × A, in two pairs of group B × B (one snail per pair) and in two pairs of group A × B (both partners alternating). During the swollen state of the head-wart the head and the anterior part of the foot was lifted above the ground. The optic tentacles were moderately contracted and directed backwards. In this position the snail bended its head alternating to the right and left (comp. Fig. 3 and 4 in Falkner 1993). In all these cases, the partners reacted with crawling to the snail with the swollen head-wart. The head-wart disappeared immediately, when snails started touching each other with tentacles or lips. However, with one exception, pairs that displayed head-warts stopped mating behaviour in P1, P2, P3 or P4.

Comparison of mating behaviour

Mating success is summarized in Table 3. Each pair was classified according to its mating behaviour into one of

Table 3. Results of mating experiments of mating groups A x A, B x B and A x B. Left number – pairs, where both partners had been in trials for the first time were included (n = 24 per group). Right number – all pairs included (n = 84 per group); values in parentheses (in third column) – percentages of successful reciprocal copulation.

	No mating	Mating terminated	Successful reciprocal copulation	n observat.
A x A	3 / 30	9 / 32	12 (50.0) / 22 (26.2)	24 / 84
B x B	14 / 29	8 / 39	2 (8.3) / 16 (19.0)	24 / 84
A x B	11 / 56	10 / 24	3 (12.5) / 4 (4.8)	24 / 84

the following three categories: no mating behaviour, terminated mating behaviour in one of the four phases (P1–P4), and reciprocal copulation (P5).

Comparing those pairs where both partners had been in trials for the first time (24 mating pairs; left numbers in Table 3), successful copulations between A × A were much more frequent than in the other two combinations. Concerning the complete lack of mating behaviour, B × B and A × B were similar (~ 50 % of the pairs), while A × A had by far the lowest value. The number of interrupted mating behaviour was similar in all three groups. The null hypothesis, that there is no significant difference in mating behaviour between the three groups A × A, B × B and A × B, was rejected in the Chi-squared test ($p < 0.01$, d.f. = 4), group A × A differs significantly from the other two.

When all mating pairs of the groups A × A, B × B, A × B were compared (altogether 84 mating pairs; right number in Table 3), A × A showed the highest copulation success, followed by B × B, while A × B had by far the lowest success. Concerning the complete lack of mating behaviour, A × A and B × B were similar, while A × B had by far the highest value. The number of interrupted mating behaviour was lowest in group A × B. Finally, the null hypothesis, that there is no significant difference in mating behaviour between the three groups A × A, B × B and A × B, was rejected in the Chi-squared test ($p < 0.0001$, d.f. = 4).

Discussion

Morphologically, the individuals of the two samples used for the study differ significantly in shell height/width ratio and manifestation of the umbilicus. The population with the globular shell shape represents the nominate form *A. a. arbustorum*. The other population with the flat and umbilicated shell represents the subspecies *A. a. styriaca* (Frauenfeld, 1868) (MolluscaBase 2021).

Mating behaviour

Five phases of courtship were described in the present investigation. Sometimes, certain phases were bypassed while other times the same phase was repeated. Since P3 was observed in all pairs that finally copulated this phase may be considered essential in courtship behaviour. The eversion and enlargement of lobes could be essential for the significance of P3 with regard to a successful copulation. The existence of the lobes was mentioned already by Paasch (1843) and the eversion of lobes during courtship is described also for some other helicids (Dorello 1924, Giusti & Lepri 1980, Giusti & Andreini 1988). Different functions were discussed: to receive stimuli during courtship (Hofmann 1923), to ensure the right position of the genital apertures immediately before mating (Giusti & Lepri 1980) and to facilitate the finding of the genital apertures for the penises (Hofmann 1923). The shape and the position of the lobes during copulation attempts (comp. description of P4) points to the last two functions. When the lobes did not have the right position and not the spoon-shaped appearance, the snails did not copulate. Lind (1976) showed in *Helix pomatia* Linnaeus, 1758, which does not possess a lobe, that touching the partner with the everted atrium leads to everting the penis of the partner and, therefore, is responsible for the synchronization of the copulation (Chung 1987, Adamo & Chase 1988). The lobe of *A. arbustorum* could have the same function as the everted atrium of *H. pomatia* during copulation attempts, yet, this appendix of the genital atrium presumably has more functions. Therefore, the neutral term "lobe" should be used rather than the term "Reizkörper" of Hofmann (1923).

Biting as a pre-copulative behaviour was observed in several helicids, e.g., *Cepaea nemoralis* (Linnaeus, 1758) and *Cepaea hortensis* (O.F. Mueller, 1774) by Peterson (1971), in *H. pomatia* by Lind (1976), and in *Helix aspersa* (O.F. Mueller, 1774)(now this species´ valid name is *Cornu aspersum*) by Chung (1987) and Adamo & Chase (1988). Nevertheless, the reason for biting remained unclear. It was assumed that the sexual motivation of the partner is tested (Giusti & Lepri 1980). In the mating experiments of *A. arbustorum* performed in the present study, no difference was observed between the three mating groups. The majority of pairs that finally mated successfully displayed biting behaviour suggesting that it is important, albeit not indispensable.

The function of the love-dart, which some Helicidae form and which is pushed into the body of the copulation partners during mating, has been discussed and interpreted differently (Meisenheimer 1907, Goddard 1962, Jeppessen 1976, Tompa 1980, 1982, Adamo & Chase 1988, 1990, Leonhard 1992) and remained unclear for a long time (Kothbauer 1988). More recent data suggest that it might influence the survival of sperms in the sperm receiving partner (Chase 2006, Chase & Blanchard 2006, Lodi & Koene 2016). The small number of observed love darts in this study could be explained by the work of Chung (1986) on *H. aspersa*. He showed a correlation

between the formation of the first love-dart of an individual and mating experience: snails without mating experience do not yet have a love dart and the formation of the first dart is initiated by the first mating attempts. Thus, the small number of love darts found in the present study may be a consequence of the experimental setting.

The milky-white viscous mucus is a secretion of the mucus glands and was secreted during P2 and P3. In the literature it was described to be often secreted simultaneously with the shooting of the love dart (Meisenheimer 1907, Hofmann 1923, Lind 1976, Chung 1987, Giusti & Lepri 1980). Such a simultaneous ejection of mucus and love dart was not observed in this study, while only two love darts were recorded, which might be explained by the lack of mating experience of the snails (see above). When mucus was ejected in the present investigation (during P3 and P4), it was frequently eaten by the snails before copula. Such a behaviour was also described in *Theba pisana* (O.F. Mueller, 1774) by Giusti & Andreini (1988). According to Bruno Baur, Basel (personal communication), the eating of the mucus in *A. arbustorum* could possibly have a stimulating effect on the partner leading to a shortened duration of copulation. Yet, eating of mucus (at least of one partner) was observed only in up to 50 % of the mating pairs (A × A; B × B) that successfully mated (Table 2). In contrast, mucus ejection (at least of one of the two partners) was observed in the majority of pairs that successfully mated. The low number of successful mating in mixed pairs (A × B), where mucus ejection and eating were observed in all those pairs, does not allow a conclusive interpretation of this behaviour in this mating group.

In some terrestrial snails a peculiar organ named head-wart exists between the bases of the optic tentacles (Taki 1935), which appears at the beginning of courtship. In the present study, head-warts were observed for the first time in *A. arbustorum* (ten individuals). Takeda & Tsuruoka (1979) suggested that the head-wart is a sex pheromone secreting gland. Further investigations proofed it to be the target organ of the steroid hormone, testosterone, secreted from the hermaphrodite gland (Takeda 1980). Falkner (1993) reported that also other species are attracted by individuals with head-warts. Therefore, it might be true that the head-wart is a decoy gland with a long-distance affect. However, the swelling of a head-wart does not always lead to copulation (comp. Takeda & Tsuruoka 1979), which is confirmed by the present results, where almost all pairs that displayed head-warts finally did not reach copula.

Prezygotic isolation

The present study was based on two populations (representing two morphotypes) originating from very distant locations. The individuals of population A originated from the lowlands near Klosterneuburg (Lower Austria), while the mountain population B originated from Gesäuse (Styria). The comparison of the frequencies of mating behaviour between the three groups A × A and B × B, A × B (Table 3) points to a possible prezygotic isolation mechanism: group A × B showed more often no mating behaviour and less copulations than pairs of both other groups (A × A and B × B), although this was the case only when comparing all pairs (including those with previous experience). Comparing only pairs without previous mating experience B × B was similarly unsuccessful as A × B (Table 3). An explanation could be that laboratory conditions might have been less favourable for the alpine population (B) which probably was adapted to lower temperatures and higher humidity. This assumption is supported by the fact that in B × B crosses many pairs failed to start mating behaviour at all (Table 3).

Conclusion

The present study provides for the first time a detailed description of the mating behaviour of *A. arbustorum*. Phases of courtship and copula and special behavioural patterns were observed in a high number of individuals in 252 mating experiments. Two different morphotypes (corresponding to the subspecies *A. a. arbustorum* and *A. a. styriaca*) were used for the experiments in various combinations, including also mixed couples. Low numbers of mating success in mixed couples led to the assumption of reproductive barriers, although the results must be considered with caution due to possible influences of laboratory conditions.

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