Effects of passive transponder used for individual marking on mating propensity in *Arianta arbustorum* (Helicidae, Gastropoda)

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Abstract

Passive transponder were used for marking snails individually and the influence of the transponder on the mating propensity of *Arianta arbustorum* was tested. There was not significant difference in the mating propensity. Nevertheless, the difference was considerable high and it is supposed that this marking method should not be applied in investigations of mating behaviour aspects.

Keywords: Gastropoda, Helicidae, *Arianta arbustorum*, individual marking, mate choice, mating propensity, transponder.

Introduction

In many studies, made in the laboratory or in the field, it is necessary to mark animals. Ideally, the marking should have no influence on the individuals (neither on their viability and longevity nor on their behaviour) and it should be permanent, readable over the period of study and, if necessary, a large number of different individuals should have to be marked individually (for assumptions in capture – recapture methods see BEGON 1979). Many methods do not meet these requirements. Especially in snails, it is very difficult to find a suitable marking method. Methods like tattooing slugs with dye (HOGAN & STEELE 1986) or brand marking slugs (RICHTER 1976) have disadvantages like high mortality and/or snails have to be checked very intensive whether they are marked or not. Using colours in marking snails, the number of different individual identifications is restricted by the number of different colours and sites. Methods like marking snails individually by writing numbers on shells with a waterproof felt tipped pen on a spot of correction fluid (BAUR & BAUR 1992) are probably not very suitable for long-term field studies. KLEEWEIN (1996) used a method introduced by WOLDA (1963) for marking *Arianta arbustorum* individually by drilling holes into the shells according to binary coded numbers. One of the most recent developments are passive transponders which can be used for individually marking. The main advantages of this method are an easy and reliable readability and a long duration (GRIMM 1996). Originally developed as a implant technique for pets and zoo animals (BEHLERT 1989), it is now also used for investigations on amphibians (SINSCH 1992, FASOLA et al. 1993) and slugs (GRIMM 1996).

The aim of the present study was to test in the course of a mate choice test, whether there is an influence on the mating propensity of individuals of *A. arbustorum* marked with transponder in comparison with unmarked individuals.

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Material and methods

Study organism

Arianta arbustorum is a simultaneously hermaphroditic helicid land snail, which is common in northern (including Iceland), western and central Europe and reaches altitudes of up to 2700 m a.s.l. (KERNEY et al. 1983). The snail has determinate growth and individuals become sexually mature at an age of 2 - 4 years (BAUR & RABOUD 1988). Cross fertilization is the rule, but self-fertilization was observed in a few virgin individuals that were kept in isolation for 2 - 3 years (CHEN 1993).

Mating in *A. arbustorum* includes elaborate courtship behaviour with optional dart shooting (dart shooting frequency is between 20 % and 40 % in natural populations; BAMINGER et al. in press) and lasts several hours (BAUMGARTNER 1997). Copulation is reciprocal and copulation duration was 121 min (95 % - confidence interval for the mean value: 116 - 127 min) in the laboratory (BAUMGARTNER 1997). After simultaneous intromission each snail transfers one spermatophore. Snails copulate repeatedly during a reproductive season, and sperm can be stored for more than 1 year (BAUR 1988). Mating was found to be random with respect to shell size in natural populations of *A. arbustorum* (BAUR & Baur 1992).

General methods

A total of 224 adult individuals of *A. arbustourm* were collected in an area of 10 x 10 m on June 3rd 1997 at the location Hundsleit'n in the Gesäuse (Styria, Austria) (47° 34' N, 14° 40' E) (comp. BAMINGER 1997) soon after snow break.

The snails were randomly separated into two groups and then the individuals of the two groups were kept individually isolated in plastic boxes (measuring 90 x 60 x 90 mm) in a cellar under natural light and temperature conditions for about 3 weeks. The bottom of each box was covered with soil and the snails were fed on lettuce and carrots *ad libitum*.

The snails thought to be marked with a transponder were weighed before the start of the mate choice – test without transponders by means of a electric scale (Sartorius 2354, d = 0.01g) to receive the initial body weight.

The mate choice – test was conducted to examine whether there is a discriminatory behaviour of snails against individuals marked with a passive transponder or not. On three snails of the group "transponder present" (TP) a passive transponder (Trovan ID 100, cylindrical glass capsule 2.2 x 11 mm; manufacturer of the transponders: Usling GmbH, Metternicherstraße 4, D - 53919 Weilerwist) was fixed. 12 hours before starting the trial. The transponder was glued on the upper side of the shell opposite to the aperture (similar the suggestion of Auffenberg 1982) with a contact glue. The transponders used do not need an energy source like a battery and therefore they have nearly an unlimited duration of life. Each transponder emits an unique ten-space alphanumerical code following activation by a scanner (Trovan LID 500). The trials of the mate choice – test were made between June 23rd and June 26th 1997 and they were always started at 11 pm. In each trial six snails, three each randomly chosen from the groups TP and "transponder absent" (TA), were placed in a transparent plastic container (measuring 260 x 190 x 70 mm), used as a test arena in regulated distances. The bottom of the containers was covered with moist paper towelling to facilitate snail activity.

Observation of the animals began immediately after the start of the trials. The snails were checked regularly and the duration of the observations was between 12 and 36 hours per trial. All snails were only used once.

Only the first copulation in a trial was used for analyses (comp. BAUR & BAUR 1992) but the observation sessions were continued until the snails were obviously indignant to copulate. Snails involved in the first copulation were used to determine the mating propensity. Assuming random mating and non-discriminatory behaviour among individuals, the probability for heterotypic pair formation is (0.5 x 0.6) + (0.5 x 0.6) = 0.6 and 2 x (0.5 x 0.4) = 0.4 for homotypic pair formation. A second kind of expected frequency of pair formation which takes differences in mating propensity into account was calculated (BAUR & BAUR 1992). Deviations from random mating were evaluated using chi-squared tests (SOKAL & ROHLF 1981).

A capture – recapture test was made at the location Hundsleit'n to test the application of the transponder in the field. 25 adult *A. arbustorum* were caught and marked with a transponder on August 3rd 1997. The test area was searched for marked snails using the Trovan scanner during four sampling occasions until October 8th 1997. Individual identification was enabled through the transponder code.

Results

The initial body weight of the snails of the group TP averaged 3.26 g (range 2.15 - 4.61, n = 108). The weight of the transponder is 0.1 g and thus the weight of a transponder is on average 3.13 % (range 2.17 - 4.65, n = 108) of the weight of a snail.

A total of 36 trials was made during 4 days using 216 snails (108 individuals each of the two groups). 32 (= 88.9 %) out of these 36 trials were successful, i.e. there was at least one copula (Table 1). In 17 out of 32 trials there was only one copula (= 53.1 %), in 14 trials there were 2 copulations (= 43.8 %) and in 1 trial there were three copulations (= 3.1 %).

In 11 (= 34.4 %) of 32 first copulations, homotypic pair formation was observed (Table 1). In 3 copulations (= 27.3 %), the homotypic pair formation was between individuals of the group TP and in 8 copulations (= 72.7 %) between individuals of the group TA. In 21 copulations (= 65.6 %) heterotypic matings were observed. Differences in the mating propensity between the two groups are not significant ($\chi^2 = 2.22$, df = 1, $P = 0.14$). Considering the first copulation of each trial, individuals of the group TA showed a higher mating propensity (= 34.3 %) than individuals of the group TP (= 25.0 %). These differences in the mating propensity result from the greater number of homotypic matings in the group TA. Pair formation did not deviate significantly from expected random mating considering the first copula of all trials ($\chi^2 = 2.38$, df = 2, $P < 0.25$) (Table 2). Pair formation did also not deviate significantly when weighing the expected frequencies for random pair formation with the mating propensities ($\chi^2 = 0.82$, df = 2, $P < 0.5$).

In the capture - recapture test, 21 of 25 marked individuals (= 84 %) were recaptured at least once and recapture rate decreased from 68 to 55 % (in % of living individuals) and from 72 to 44 % (in % of marked individuals) (Table 3). Four snails died during the investigation period and one snail lost the transponder.

Tab. 1: Homotypic (TPTP, TATA) and heterotypic (TPTA) copulations (X) in marked (TP) and unmarked (TA) individuals of *Arianta arbustorum*. Bold numbers indicate missing copulation in this trial.

		First copula			Second copula			Third copula		
Day	Trial	TPTP	TPTA	TATA	TPTP	TPTA	TATA	TPTP	TPTA	TATA
	1		X							
	2		X							
	3		X							
	4		X							
1	5			X		X				
	6		X							
	7			X		X				
	8	X				X				X
	9			X		X				
	10		X							
	11		X				X			
	12	X								
	13		X							
2	14		X			X				
	15									
	16	X					X			
	17		X				X			
	18		X							
	19									
	20									
	21			X						
	22		X							
3	23		X							
	24		X		X					
	25		X			X				
	26		X							
	27			X	X					
	28			X	X					
	29			X						
	30			X		X				
	31		X			X				
4	32		X							
	33		X							
	34									
	35		X							
	36		X				X			
		3	21	8	3	8	4	-	-	1

Discussion

Using techniques of tagging like the fixation of transponder/radio-transmitter, the relation between body weight and weight of the transponder is of great importance. For a successful and reliable work, there should be no influence on the investigation objects

by taggers. The use of to heavy taggers can result in decreased longevity and vitality or reduced dispersal and mating propensity. In land snails, AUFFENBERG (1982) first used the radio tracking technique for *Ryssota uranus* (Pfeiffer). The total weight of transmitter and battery was about 30% of the body weight and TOMIYAMA & NAKANE (1993) stated, that "… this may have strongly influenced the behaviour …". BAILEY (1989) used a radio-transmitter, which weighed about 25% of the snails body weight for studies of the locomotion of *Helix aspersa* Müller. TOMIYAMA & NAKANE (1993) used a micro radio-transmitter which weighed about 5 to 10 % of the body weight of *Achatina fulica* and they did not mention any disadvantages of his method concerning behavioural aspects. In the present study, the weight of the passive transponders is about 2 to 5 % of the body weight. Using a tagger with minor weight in relation to the body weight than those mentioned above, one can assume that there is, like in the study of TOMIYAMA & NAKANE (1993), no influence on an individual´s behaviour caused by the tagger´s weight.

The multiple choice design was chosen because field observations indicate that this design may represent a close approximation to the natural conditions of courtship and mating. In *H. pomatia* courting groups of snails involving several individuals have frequently been observed under natural conditions. LIND (1988) stated, that in 9 % of the observed copulations more than 2 individuals (up to 5 individuals) took part in precopulatory behaviour. In *A. arbustorum*, groups of 3 individuals were observed several times in the field by the author and during the mate-choice tests groups of up to 4 individuals were observed taking part in precopulatory behaviour.

Only the first mating pair of each mate choice trial was considered to get informations about a possibly existing discriminatory behaviour of potential mating partners against transponder-marked individuals, because in subsequent matings pair formation was influenced by different numbers of partners from the two groups available. The results of the mate choice test showed that pair formation in the first copula did not deviate significantly from random mating, i.e. individuals of *A. arbustorum* met homotypic and heterotypic mates in frequencies expected by chance. Weighing the expected frequencies for random mating pair formation with the mating propensities, the pair formation in the first copula did also not deviate significantly from random mating. However, there was a greater number of homotypic matings in snails of the group TA than in the group TP. Baur & Baur (1992) explained differences in the frequency of homotypic matings in *A. arbustorum* as "… partly derived from the fact that snails of one population simply mated more frequently than did the snails of the other population." In the present study, this explanation is unsuitable because individuals of the two groups are from the same population. There was no difference in the activity of the snails of the two groups at the begin of the trials and therefore this has to be excluded as an explanation for differences in homotypic matings. The larger number of homotypic matings within the group TA and thus the higher mating propensity of the individuals of the group TA can possibly be explained through the mating behaviour of *A. arbustorum*. The mating behaviour of land snails is often very complicated (*Helix aspersa*: ADAMO & CHASE 1988, CHUNG 1987; Partula: LIPTON & MURRAY 1979; *Achatina fulica*: TOMIYAMA 1994; *Helix pomatia*: LIND 1976, JEPPESEN 1976; *Theba pisana* and *Helix aperta*: GIUSTI & ANDREINI 1988). For *A. arbustorum* a very detailed description was given by BAUMGARTNER (1997). During the precopulatory behaviour, individuals of *A. arbustorum* turn around each other and sometimes they also crawl one on the top of the other. The presence of a transponder may irritate some snails in that way that they interrupt courtship with marked

Tab. 2: Mate-choice test with marked (TP) and unmarked (TA) individuals of *A. arbustorum.*

Number of matings	Mating pairs	Observed number of matings	Expected for random mating	$\chi 2$	P	Expected matings weighted with mating propensity	$\chi 2$	P
32	TP x TP	3	6,4			4,4		
	TP x TA	21	19,2			18,7		
	TA x TA	8	6,4	2,38	< 0.25	8,9	0,82	< 0.5

Tab. 3: Capture - recapture in marked individuals of *Arianta arbustorum.* X = recaptured, – = not recaptured; * = transponder found on dead snail; ** = only transponder found.

Individual	Recapture 1	Recapture 2	Recapture 3	Recapture 4
00-0120-F9E1	–	–	–	–
00-0120-F7EC	X	X	X	X
00-0121-4495	X	X	X	–
00-0121-5A4D	X	–	X	X
00-0121-51D4	–	–	–	–
00-0121-4956	X *	–	-	–
00-0121-3D10	X	X	X *	–
00-0121-4D0D	X	–	X	X
00-0121-59B4	X	X **	–	–
00-0121-3DBF	X	X	–	X
00-0121-493E	–	X	–	–
00-0121-5235	–	X *	–	–
00-0121-5031	X	X	X	X
00-0121-3EFC	X	X	–	–
00-0121-3DE1	X	X	X	X
00-012E-60FF	–	X	X	X
00-012E-6275	X	X	–	–
00-012E-5DB5	X	X	X	X
00-0134-394F	X	X	X *	–
00-0134-319C	X	–	X	X
00-0134-3C11	–	–	–	–
00-0134-335F	–	–	–	–
00-0134-3229	X	X	–	–
00-0134-3F3A	X	X	X	X
00-0135-B543	X	X	X	X
individuals recaptured in % of living individuals	68,0	62,5	50,0	55,0
individuals recaptured in % of marked individuals	72,0	68,0	52,0	44,0

individuals and change to unmarked ones or the marked individuals themselves are irritated by the transponder and this irritation leads to a lower mating propensity.

Durability of fixation of transponders was checked under natural conditions. Only one of 25 marked individuals had lost the transponder during the investigation period. Fixation *per*

se is satisfying but the rather high mortality (4 of 25 individuals) points out that the influence of the fixed transponders on the "daily routine" of the snails is possibly considerable. The use of transponder for individual identification is generally very satisfying but the scanner is, because of his low activity radius, not very helpful in finding marked individuals.

To summarise, mate-choice tests in *A. arbustorum* showed no significant influence of the transponder on the mating propensity. Nevertheless, differences in mating propensity are still considerably high and for this reason this method of marking snails individually should not be used in experiments where affects on behaviour are disadvantageous. Suitability of the method for experiments in the field (dispersal, activity) has to be tested.

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