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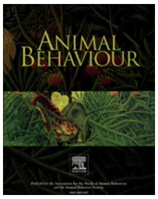

Determinants of female and male reproductive success in a simultaneous hermaphrodite land snail

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Classical sexual selection theory assumes that the reproductive success of females is limited by the resources available for egg production, while the reproductive success of males is determined by the number of mates (Bateman's principle). It has been suggested that the optimal mating rates should also diverge between gender functions within individuals of simultaneous hermaphrodites. We assessed determinants of mating success and female and male reproductive success in individuals of the simultaneous hermaphrodite land snail *Arianta arbustorum*. We videorecorded the behaviour of individually tagged snails kept in groups of six animals over one reproductive period (58 days) and assigned the genotyped hatchlings to the female and male function of individual parents. We found considerable interindividual variation in the activity of snails, which is a combined measure of time spent crawling, feeding and digging. The snails mated between zero and three times. Mating success, which is equal to the female and male function in simultaneous hermaphrodites with reciprocal copulation, was mainly determined by the activity of an individual. We found that female reproductive success (number of hatchlings emerging from the eggs laid by the focal snail) was positively correlated with male reproductive success (number of hatchlings sired by the focal snail) and that both were determined by the individual's activity. Furthermore, both female and male reproductive success of an individual were influenced positively by the snail's degree of genetic heterozygosity and negatively by shell size. Our results challenge the trade-off assumption of sex allocation theory in simultaneous hermaphrodites.

Much recent research effort has been directed at explaining determinants of the reproductive success of females and males in gonochoristic animals (Clutton-Brock 1988; Roff 2002). In mating systems without paternal care, male fitness tends to be more tightly linked to mating success than is female fitness (Trivers 1972). This can be explained by Bateman's principle, which states that the female's reproductive success is primarily limited by the energy available for producing gametes, whereas the reproductive output of males is primarily governed by the number of mates (Bateman 1948). However, in species in which individuals are promiscuous, sexual selection continues after copulation in the forms of sperm competition and female manipulation of sperm. Sperm competition occurs when spermatozoa from different males compete in the reproductive tract of a female for the fertilization of her eggs (Parker 1970). In a variety of species, females have a physiologically and morphologically complex reproductive system, which may enable them to control or influence offspring paternity by postcopulatory sperm storage and selective sperm use (Eberhard 1996). There is increasing evidence that females choose between sperm from different males after copulation and that this so-called cryptic female choice may also affect paternity (Pitnick et al. 2009).

Simultaneous hermaphrodites are functional female and male at the same time. This type of gender expression is widespread in the animal kingdom and among plants (Michiels 1998; Jarne & Auld 2006). Bateman's principle also applies to hermaphrodites (Charnov 1979; Anthes et al. 2010). Despite the central role of number of mates in sexual selection theory, only very little is known of the absolute number of mating partners simultaneous hermaphrodites can acquire and how this number varies between individuals. For example, laboratory experiments with the hermaphrodite flatworm *Macrostomum lignano* revealed considerable variation in the number of mates and in sperm transfer success between individuals (Janicke & Schärer 2009). The number of matings increased with group size. However, food availability, and not group size, had a significant effect on female fecundity (Janicke et al. 2011). Furthermore, our knowledge of factors responsible for the variation in reproductive success via either sex function is still

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limited. The optimal mating rates may diverge between sex functions within an individual (Anthes et al. 2006). The possibility of a flexible division of resources between male and female functions as well as conflicts between individuals over sexual roles may further complicate sexual strategies in simultaneous hermaphrodites (Schärer 2009). It has been suggested that having both sexes combined in the same individual may limit sexual selection for traits involved in mate acquisition (Greeff & Michiels 1999). However, other features, such as multiple mating, sperm storage and internal fertilization, leading to traits for sperm competition and cryptic female choice, may be similar in simultaneous hermaphrodites and gonochoristic species (Baur 1998; Michiels 1998).

Research on mating strategies in simultaneous hermaphrodite land snails has been hampered by the notorious difficulty with which mating can be reliably observed in natural populations. Evidence for promiscuity and multiple paternity in broods is available for several snail species. Individuals of *Helix pomatia*, *Cornu aspersum*, *Cepaea nemoralis* and *Arianta arbustorum* have been observed to mate repeatedly with different partners in the course of a reproductive season resulting in multiple-sired broods (Wolda 1963; Baur 1988a, 1994a; Lind 1988; Rogers & Chase 2002; Evanno et al. 2005; Kupfernagel et al. 2010). However, little attention has been devoted to the fitness consequences of multiple mating in land snails (Jordaens et al. 2007).

We used a combination of behavioural and genetic data to examine the factors that influence female and male reproductive success in individuals of the simultaneous hermaphrodite land snail *A. arbustorum*. We kept animals in groups in a seminatural environment and videorecorded the behaviour of the individually tagged snails over one reproductive season. Using microsatellite markers, we genotyped the emerging hatchlings and assigned the offspring to the female and male functions of individual parents. We also examined the potential influence of heterozygosity of a parent snail on its female and male reproductive success because there is evidence in a variety of species that the fitness of an individual increases with increasing degree of heterozygosity (David 1998; Markert et al. 2004). As egg production is assumed to be more resource limited than sperm production in our model species, we predicted that male reproductive success would increase with increasing number of matings, while female reproductive success would level off with additional copulations. Furthermore, we asked which factors (behavioural traits, shell size, level of heterozygosity) determine mating and reproductive success in each sex function in individuals of *A. arbustorum*, and whether our results support existing models of sexual strategies in hermaphrodites.

METHODS

Study Species

Arianta arbustorum is common in moist habitats of northwestern and central Europe (Kerney & Cameron 1979). The snail has determinate growth (shell breadth of adults 17–22 mm). Individuals become sexually mature at 2–4 years, and adults live another 3–4 years (maximum 14 years; Baur & Raboud 1988). In the field, snails deposit one to three egg batches consisting of 20–50 eggs, per reproductive season (Baur 1990). Breeding experiments showed that 27% of virgin snails prevented from mating produced a few hatchlings by self-fertilization in the second and third years of isolation (Chen 1993). The reproductive success of selfing individuals, however, is less than 2% of that of mated snails, suggesting high costs for selfing (Chen 1994).

Mating in *A. arbustorum* includes elaborate courtship behaviour with optional dart shooting (i.e. the pushing of a calcareous dart into the mating partner's body), and lasts 2–8 h (Baur 1992). Copulation is reciprocal. After intromission, each snail simultaneously transfers one spermatophore (Haase & Baur 1995). The spermatophore is formed and filled with sperm during copulation (Hofmann 1923). It has a distinctive form consisting of a head, a body (sperm container with 800 000–4 000 000 spermatozoa) and a tail 2–3 cm long (Baur et al. 1998). Fertile sperm can be stored for more than 1 year (Baur 1988a). Mating is random with respect to shell size and different degrees of relatedness (Baur 1992; Baur & Baur 1997). Snails need at least 8 days to replenish their sperm reserves after a successful copulation (Locher & Baur 1999; Hänggi et al. 2002).

Paternity analyses in broods of wild-caught *A. arbustorum* revealed a high frequency of multiple inseminations (Baur 1994b; Kupfernagel et al. 2010). A controlled laboratory experiment showed that one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by individual snails kept singly (Chen & Baur 1993). However, there is a probability of 5–8% that copulation will not lead to fertilization of eggs (no sperm transfer or transfer of infertile sperm; Chen & Baur 1993).

Experimental Animals

To obtain virgin snails we collected subadult individuals that had not yet completed shell growth from an embankment along a track in a subalpine forest near Gurnigelbad, 30 km south of Bern, Switzerland (46°45′N, 7°28′E) at an altitude of 1250 m above sea level. We kept the snails isolated in transparent beakers (8 cm deep, 6.5 cm in diameter) lined with moist soil (approximately 4 cm) at 19 °C and on a light:dark cycle of 16:8 h for 5 weeks. They were fed fresh lettuce ad libitum. During this period, subadult individuals reached sexual maturity as indicated by the formation of a flanged lip at the shell aperture. We marked the snails individually on their shells with symbols and lines drawn with correction fluid (Tipp-Ex). The animals showed no visible reaction to the marking procedure. We also measured the shell width of each snail to the nearest 0.1 mm using vernier callipers immediately before the experiment.

Video Tracking

We constructed observation chambers that allowed (1) continuous recording of snails over the entire reproductive period; (2) free movements of animals on natural substrate (soil); (3) egg deposition; (4) continuous identification of individual snails; and with (5) a seminatural temperature regime and a light:dark cycle of 16:8 h.

We kept groups of six randomly chosen snails in each observation chamber, a transparent plastic box measuring 29 × 19 cm and 17 cm high, lined with a 4 cm thick layer of moist soil and covered with a glass plate. Light was provided by cold-light sources (Osram Dulux L). A window kept constantly open allowed daily air temperature fluctuations. The room temperature varied from 16.5 to 24.1 °C (mean 22.1 °C). Fresh lettuce cut into small pieces was provided twice a week. Eight observation chambers with a total of 48 snails were arranged in a line.

To record the behaviour of the snails we used a computerized video-image technique. We installed a SONY camcorder with infrared illumination 80 cm perpendicular to each observation chamber. The eight cameras were connected via a Kramer VS-2081S 8x1 S-video switcher to a computer, which recorded a video frame from each camera at an interval of 2 min. As snails move slowly, this frame interval gave a reasonably accurate representation of the various behavioural elements. We recorded the snails' behaviour nonstop over a period of 58 days from 21 June to 18 August 2000. There were a few short gaps in recording owing to technical problems with the computer. Overall, data collected