Promiscuity and sex ratio in the terrestrial isopod Armadillidium vulgare and consequences on genetic diversity

Sylvine Durand, Christine Braquart-Varnier, Sophie Beltran-Bech

▶ **To cite this version:**

Sylvine Durand, Christine Braquart-Varnier, Sophie Beltran-Bech. Promiscuity and sex ratio in the terrestrial isopod Armadillidium vulgare and consequences on genetic diversity. Behavioural Processes, 2020, 171, pp.104030. 10.1016/j.beproc.2019.104030 . hal-03111010

HAL Id: hal-03111010
https://hal.science/hal-03111010

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S0376635719304498
Manuscript_ddeb8798341db2091de57ed4cc31a1fb

Promiscuity and sex-ratio in the terrestrial isopod *Armadillidium vulgare* and consequences on genetic diversity

Sylvine Durand, Christine Braquart-Varnier, Sophie Beltran-Bech

Laboratoire Ecologie et Biologie des Interactions, Equipe Ecologie, Evolution, Symbiose, Université de Poitiers, UMR CNRS 7267, F-86073 POITIERS, France

Telephone: +33 5 49 36 64 08

Fax: +33 5 49 45 40 15

Corresponding author: Sylvine Durand (durand.sylvine@gmail.com)

Abstract:

In promiscuous mating systems, both males and females mate with several partners. While the benefits of multiple mating are well recognized for males, there are several non-mutually exclusive hypotheses to explain multiple mating for females. Promiscuity is widespread in terrestrial isopods. Here, we placed experimental populations of the terrestrial isopod *Armadillidium vulgare* under varying sex-ratios to manipulate the number of available partners, and better characterise the mating system in this species by performing paternity tests using microsatellite markers. We observed that females usually mate multiply with up to 5 males in a single event of reproduction. A higher number of fathers in broods did not increase brood size nor heterozygosity, but increased allelic richness. Promiscuity seems to be asymmetric in this species, with females being less affected by changes in sex-ratio than were males and would be an adaptive mating system maximising the offspring genetic diversity.

Keywords: crustaceans, multiple paternity, promiscuity, sex-ratio bias

© 2019 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

Introduction

Unlike polygamy, where only one of the two sexes mates with several partners (the female in the case of polyandry and the male in polygyny), promiscuity, also called polygynandry, is a mating system in which individuals of both sexes mate with several partners during the same breeding period. The question of the benefits of multiple mating for both sexes has been studied (Reynolds 1996; Jennions and Petrie 2000). For males, these advantages are obvious, since the number of offspring increases with mating success and therefore with the number of partners (Bateman 1948). For females, however, the benefits of multiple mating remain uncertain, as a single male is usually sufficient to fertilize all oocytes. Two types of advantages resulting from multiple mating have been proposed for females: (i) direct benefits resulting from resources provided by males to females or offspring (Arnqvist and Nilsson 2000), such as food or protection, and (ii) indirect benefits, also known as genetic benefits, leading to better genetic quality in offspring (Yasui 1998; Jennions and Petrie 2000), such as inbreeding avoidance or increased allelic diversity in broods.

In addition to these advantages, multiple mating increases costs associated with mating (Pomiankowski 1987), such as the risk of predation during partner search and mating (Rowe 1994), the risk of injury during mating (Chapman et al. 1995) and the risk of contracting a sexually transmitted disease (Thrall et al. 2000). Despite these costs, the observation of multiple paternity in broods in many vertebrate species (fish (Coleman and Jones 2011), amphibians (Adams et al. 2005), reptiles (Uller and Olsson 2008), birds (Griffith et al. 2002), mammals (Avise et al. 2011)) but also in invertebrates (insects (Simmons et al. 2007), molluscs (Wacker et al. 2018), crustaceans (Walker et al. 2002; Yue and Chang 2010; Jossart et al. 2014)) reveals that multiple mating in females is quite widespread.

When it comes to mating systems in terrestrial isopods, monogamy is rare. A few cases have been recorded within the genus *Hemilepistus* (Trachelipodidae) (Lefebvre 2002), this mating system resulting from a need for biparental care (burrow digging, offspring care) in relation to the arid and desert environmental conditions under which these organisms live. An intermediate step towards the evolution of a monogamous system would also be reported for species of another family (Porcellionidae, genus *Porcellio*) even if the majority of terrestrial isopods are promiscuous (Lefebvre

2002). Indeed, in many species, both males and females are able to mate with several species (Lefebvre 2002). And in these cases, as expected, parental care is provided exclusively by the female (Krebs and Davies 1997).

In *Armadillidium vulgare* (as in terrestrial isopods in general), multiple paternity is facilitated by to the presence of a seminal receptacle in females that can store sperm after mating (Suzuki and Ziegler 2005; Ziegler and Suzuki 2011). Stored sperm can be used to produce offspring even after females have mated with and received sperm from another male (Moreau et al. 2002). However, because of this sperm storage capacity, a single mating is sufficient to fertilize all the oocytes produced for a year (Howard 1943) and even throughout the life of a given female (Beltran-Bech S., personal observation), which raises the question of the evolution of promiscuity in this species and the direct or indirect benefits that this promiscuity mating system can bring to reproductive partners.

In this species, multiple paternity has been observed in laboratory experiments (Moreau et al. 2002; Verne et al. 2007; Durand et al. 2017; Bech et al. 2017) when two males were proposed to a same female (simultaneously or sequentially), and in a natural population in which all of the 23 tested broods presented multiple paternity, with an average of 4.48±1.24 fathers per brood (Valette et al. 2017). This clearly shows that experimental observations of multiple paternity in the laboratory are not an artefact and that in this species, the mating strategy is promiscuity. However, the study on a natural population did not allow to access to the number of males encountered by females, or to the timing in which mating took place (in a single or in multiple breeding events, *A. vulgare* females usually producing two to three broods per year between April and October (Vandel 1962)). In particular, it is currently unknown how the availability of sexual partners (e.g. sex ratio) affects the propensity of females to mate.

Wishing both to simulate more natural conditions in our experiments and to test the effect of sex ratio on the intensity of multiple paternity in *A. vulgare*, we placed experimental populations under three different sex ratio conditions to measure the effect of the number of present reproductive partners on the number of fathers per brood during the same reproductive event, using microsatellite markers to perform paternity tests. These markers also allowed us to estimate the level of heterozygosity and allelic richness of broods as a function of the number of fathers in these broods.

Material and methods

1. Ethical statement

The European Directive 2010/63/EU and the French decree n°2013-118 regulating animal research do not require ethical evaluation prior to research on arthropods. However, we complied with the 3R ethical rules: even though replacement was not possible, sample size was not disproportionate and we took care in providing our animals with food and suitable living conditions during the whole duration of the experiment. Tissue sampling for adult genotyping was not lethal, and offspring that had to be killed were frozen before DNA extraction. After the end of the experiment, surviving animals were placed together in large boxes under standard rearing conditions.

2. Experimental setup

We used 120 one-year-old, virgin, sexually mature individuals in our experiment (48 males and 72 females) belonging to a laboratory lineage descending from animals collected in 1982 in Helsingör, Denmark. This lineage is free from the endosymbiont *Wolbachia*, known to affect *A. vulgare* biology, including sexual behavior (Moreau et al. 2001). A leg was collected from each individual using thin tweezers right before the experiment to perform subsequent genetic analyses.

Animals were gathered into twelve experimental populations of ten animals, distributed into four sex-ratio conditions each with three replicates: (i) male-biased (2 females and 8 males, i.e. 20% of females, hereafter $20\%_F$), (ii) balanced (5 females and 5 males, $50\%_F$), (iii) female-biased (8 females and 2 males, $80\%_F$) and (iv) female-biased with 9 females and 1 male ($90\%_F$) as a control to determine how many females a single male could inseminate within the duration of the experiment in the absence of male-male competition. We did not place siblings or cousins in the same population to avoid close inbreeding avoidance or kin selection mechanisms. Animals were placed in boxes filled with humid loam, with dried linden leaves and carrot slices for food, and placed under a stimulating photoperiod (18L: 6D) to enhance reproduction.

Once a week after the beginning of the experiment, boxes were searched for all animals to monitor death and female reproductive status: after mating, females lay fertilized eggs in the *marsupium*, a

ventral pouch which appears after moulting. Fully developed offspring leave the *marsupium* after one month (Surbida and Wright 2001). All females (except one) had undergone a parturial moult after three weeks of experiment, so males were removed to avoid disturbing females during incubation. Three weeks after the first parturial moult within a population (i.e. five or six weeks after the beginning of the experiment), all females were individualized then controlled daily, and all maternal siblings were counted on their day of birth or the following day. The number of offspring collected for molecular analyses depended on sex-ratio conditions: the more males are present in a population, the more fathers are expected to share paternity in a brood, and the more analyzed offspring are needed to increase our power to detect all fathers. We thus collected 40 offspring for $20\%_F$, 30 offspring for $50\%_F$ and 20 offspring for $80\%_F$ populations. For $90\%_F$ populations, no paternity analyses were required with only one possible father, but 15 offspring were collected from three randomly selected broods in each population (hence nine $90\%_F$ broods collected) for estimation of brood genetic diversity under this control condition.

3. Genotyping

DNA was extracted individually from legs of all 120 adult using a phenol-chloroform extraction (Kocher et al. 1989; Durand et al. 2015) and from 1145 whole offspring using a Chelex (Sigma-Aldrich) extraction (Durand et al. 2017). Eleven microsatellite markers (Av1, Av2, Av3, Av4, Av5, Av6, Av9 (Verne et al. 2006); Av18, Av32 Av56, Av63 (Giraud et al. 2013)) were gathered into three multiplexes and amplified in the same conditions as in Durand et al. (2015) with fluorescein-marked forward primers. PCR products separation was performed by GenoScreen (Lille, France) on an automated sequencer. Resulting electropherograms were analyzed with the software GeneMapper version 3.7 (Applied Biosystems) to score microsatellite alleles.

4. Paternity analyses

We used the software COLONY v.2.0.6.3 to identify the parents of each offspring. Analyses were performed using a genotyping error rate of 0.01 for all loci, including only offspring for which at least three loci were genotyped (N = 1078 offspring). Genotypes of all offspring in a given brood were

entered into the software, as well as genotypes of all candidate mothers and fathers for the considered brood (i.e. all adults within the population), and we specified that all offspring shared the same mother (as well as the same father for 90%$_F$ broods). We decided to consider only offspring whose father was identified with a probability higher than 0.75 (N = 958 offspring, i.e. 88.86% of genotyped offspring), as in other studies (Coetzer et al. 2017).

For each offspring, we computed individual genetic diversity using the function GenHet (Coulon 2010) in the software R (R Development Core Team 2013). As some individuals presented missing genotypes for some loci (mean ± standard deviation = 9.38 ± 2.70 genotyped loci per individual), we computed the multilocus standardized heterozygosity H_s (Coltman et al. 1999) (hereafter named "heterozygosity") which divides the proportion of typed heterozygous loci by the mean observed heterozygosity for typed loci. For each brood, allelic richness was computed using the software FSTAT (Goudet 1995).

5. Statistical analyses

5.1. Preliminary adjustments and investigations

Two males from different populations died within the first week of experiment. Their death was attested by the observation of remains for both individuals, confirming that we did not just simply fail to detect a living animal when searching loam. In each of those two populations, all males except one produced offspring with at least one female. It is reasonable to conjecture that the two males died before having any opportunity to mate and that sex-ratio was modified at the beginning of the experiment. Therefore, we adapted our analyses to the death of those two males. (i) One of them belonged to a population with 50%$_F$. This population was analysed normally alongside with the two other 50%$_F$ populations (creating a new category with 55.6% females would not make sense with a single replicate). (ii) The second male belonged to a population with 80%$_F$, leading to a population with a single male. This population was thus treated as a fourth 90%$_F$ (control) population, leaving only two 80%$_F$ populations

5.2. Promiscuity description

Mann-Whitney tests were used to compare the number of reproductive partners between males and females according to the rarity of the opposite sex (i.e. in excess, balanced or in deficit). To compare the number of reproductive partners between the different sex-ratio conditions within each sex, we performed two Kruskal-Wallis tests (for males and for females) followed by Dunn test with Holm-Šidák adjustment for pairwise comparisons.

5.3. Brood characteristics

We modeled three brood characteristics as a function of the number of fathers in the brood: (i) brood size, using a GLMM with a log link and a variance given by a negative binomial distribution; (ii) mean brood heterozygosity and (iii) brood allelic richness using LMMs. In all cases, population identity nested within sex-ratio was included as a random effect. For the model with brood size as a function of the number of fathers, all females in $90\%_F$ populations were included, as genotyping the offspring was not necessary to obtain the number of fathers (always 1 in this case). Significance of variables was tested using type II Wald χ^2-tests.

The software R v.3.4.1 (R Development Core Team 2013) was used to perform statistical analyses. LMMs and GLMMs were realized with the functions "lmer" and "glmer" from the package "lme4" (Bates et al. 2014), excepted for models with brood size as a response variable, which were realized with the function "glmer.nb" of the same package. Type II Wald χ^2-tests were performed with "Anova" from the "car" package (Fox et al. 2017). Dunn tests were performed with the "dunn.test" package (Dinno 2016). Significance threshold was set to 0.05 and means are given ± standard error (se).

Results

1. General overview

Out of the 72 females used, 6 died without having offspring (1 in 80%$_F$ and 5 in 90%$_F$ populations), 1 never presented a *marsupium* (in 80%$_F$) and 2 produced developed embryos that were dead before birth (stillborn broods, in 80%$_F$ and 90%$_F$). Three females produced a low number of offspring (6 offspring for 2 females in 50%$_F$ and 1 female in 80%$_F$) which is way below the usual brood size (Table 1) and were discarded. The remaining 60 females produced a brood (6 females in 20%$_F$, 13 in 50%$_F$, 12 in 80%$_F$ and 29 in 90%$_F$), from which a total of 1145 offspring were genotyped (15 to 40 offspring per brood from 47 analyzed broods, as 13 90%$_F$ broods were not genotyped), among which 958 were assigned a father with a probability higher than 0.75.

2. Mating system

All surviving females in 90%$_F$ populations produced a brood (or stillborn brood), confirming that males are not limited by their capacity to inseminate all available females (up to 8 within a replicate). Moreover, up to 5 fathers were assigned for a single brood in a 20%$_F$ population, confirming the propensity of females to mate with several males during only one breeding event (Supplementary Material 1).

All males in 80%$_F$ populations reproduced at least once (Table 1), and a single male did not reproduce in a 50%$_F$ population. However, under male-biased conditions (20%$_F$), only 62.5% of males produced offspring (Table 1, Supplementary Material 2).

Multiple mating was observed for both sexes, with an intensity varying according to both sex and sex-ratio (Figure 1). The number of reproductive partners depended on sex-ratio in both females (Kruskal-Wallis KW = 15.59, df = 2, $p < 0.001$) and males (Kruskal-Wallis, KW = 21.96, df = 2, $p < 0.001$), with fewer partners when the opposite sex was in deficit than in excess or under balanced conditions, but no difference between balanced and excess conditions (number of partners for females, Dunn test: deficit – balanced p = 0.0012, deficit – excess p = 0.001, balanced – excess p = 0.209; number of partners for males, Dunn test: deficit – balanced $p < 0.001$, deficit – excess $p < 0.001$, balanced –

209 excess p = 0.054). While males and females did not differ significantly in the number of mates when
210 the opposite sex was in excess (U = 6, p = 0.21, n = 10) or balanced (U = 94.5, p = 0.88, n = 27),
211 females had more mates than did males when the opposite sex was rare (U = 231, p = 0.014, n = 36).

212

213

Table 1 Summary of mean adult genetic characteristics and reproduction-related variables for each sex-ratio condition.

Sex-ratio	% of ♂ reproducing at least once	% of broods fathered per ♂[a]	% of offspring sired within each brood per ♂[a]	Brood size[b]	Brood allelic richness	Mean brood heterozygosity
$20\%_F$ (N =3)	62.5 ± 10.10	41.67 ± 7.77	30.00 ± 3.72	158.33 ± 14.63	1.5 ± 0.01	1.07 ± 0.07
$50\%_F$ (N =3)	92.86 ± 7.14	58.21 ± 7.41	37.14 ± 3.00	124.69 ± 9.88	1.45 ± 0.01	0.94 ± 0.03
$80\%_F$ (N =2)	100 ± 0	75.00 ± 8.33	66.67 ± 8.64	123.67 ± 13.42	1.4 ± 0.02	0.97 ± 0.07
$90\%_F$ (N =4)	100 ± 0	100 ± 0	100 ± 0	126.88 ± 11.72	1.4 ± 0.02	0.96 ± 0.04
overall (N =12)	78.26 ± 6.15	54.67 ± 5.35	52.81 ± 3.52	129.47 ± 6.24	1.43 ± 0.01	0.97 ± 0.03

[a] Values have been calculated only on reproducing males, which is why the value sometimes exceeds 50%.

[b] When including 13 additional broods in $90\%_F$ populations that were not genotyped, $90\%_F$ mean brood size is 129.07 ± 9.81, and overall mean brood size is 129.97 ± 6.04.

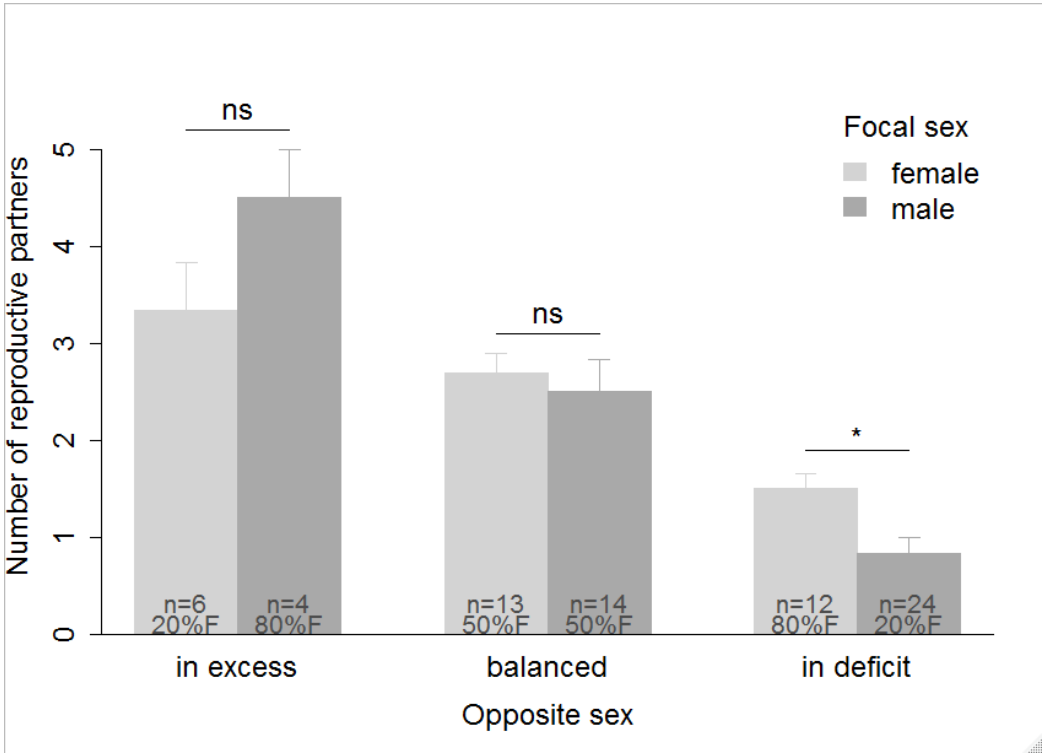

Figure 1 Mean number of reproductive partners for each sex according to sex-ratio condition. Error bars represent standard errors. Population sex-ratio is indicated in each bar. n = sample size for the focal sex. ns = non-significant difference, * = significant difference.

222 **3. Brood characteristics**

223

224 For all populations (including 13 $90\%_F$ broods which were not genotyped), the number of offspring

225 was not significantly affected by the number of fathers (χ^2=0.280, df=1, p=0.597, n=60 females).

226 While mean brood heterozygosity did not depend on the number of fathers (χ^2=0.202, df=1, p=0. 653,

227 n=47 females), a high number of fathers lead to a higher allelic richness (χ^2=5.756, df=1, p=0.016,

228 n=47 females, $\beta = 0.026 \pm 0.011$).

229

Discussion

In this study, we placed experimental populations of ten *Armadillidium vulgare* individuals under different sex-ratio conditions to estimate rates of multiple mating for both sexes and better describe the promiscuous mating system in this species.

All surviving females hosted with a single male became gravid and produced a brood (or much less frequently stillborn brood), which confirms that males are polygynous and not limited by their ability to inseminate up to 8 females in a single reproductive event. However, multiple mating may still be costly for males, as sperm depletion has been observed in this species (Rigaud and Moreau 2004). This depletion has been shown to decrease female fertility when females were infected by the endosymbiont *Wolbachia.* Under female-biased and balanced sex-ratio conditions, all males but one reproduced at least once, whereas only 62.5% of males did so under male-biased conditions: this suggests that males are limited in their access to females when these are rare. It then appears that sex-ratio affects the intensity of multiple paternity: all males reproduce but paternity is shared between few fathers within each brood when sex-ratio is female-biased, while not all males reproduce but more fathers share paternity in the opposite situation. Thus, female choice for male characteristics and/or competition between males seem to take place under male-biased sex-ratio. Lefebvre et al. (2000) have shown that in *A. vulgare*, the long antennae present in males are not used in male contests as in other terrestrial isopod species. Male-male competition seems to be based on their ability to successfully locate females (scramble competition) rather than monopolizing mates or excluding other males from reproduction. This suggests that the lower access to paternity under male-biased conditions is more the result of a female refractory behaviour than direct contest competition between males.

On the female side, polyandry was largely observed during a single reproductive event with up to 5 fathers per brood. A study on a natural population revealed an average of 4.48 fathers per brood with up to 7 fathers (Valette et al. 2017), in line with the multiple breeding events in the wild, the possibility for females to store sperm from previously encountered males (Howard 1943; Verne et al. 2007), and a strong copulatory capacity for females. The number of fathers was predictably higher under male-biased or balanced conditions than under female biased conditions, with a trend for more

fathers when males were in excess than when sex-ratio was balanced. Broods of more polyandrous females were more polymorphic but did not present more offspring, in line with previous observations (Durand et al. 2017). Interestingly, in a recent study, females hosted under different sex-ratio conditions did not differ in the number of offspring they produced per month over six months, but a lower female survival was observed under male-biased sex-ratio (Fortin et al. 2019). This suggests the existence of female costs associated with polyandry, potentially as a result of male harassment, and raises the question of the evolution of polyandry in this species. Anyhow, by allowing more males to get access to reproduction, polyandry contributes to the persistence of genetic diversity in populations (Sugg and Chesser 1994).

Our study highlighted an asymmetric promiscuous mating system in *A. vulgare*, with males being more impacted than females by sex-ratio variations. Indeed, they were more affected by a restriction in sexual partners, with significantly fewer mates per male than per female, and some males not even accessing to reproduction. This is expected since sexual selection is usually stronger in males than in females in most species. In wild populations, sex-ratio varies widely (38 to 81% females, (Rigaud et al. 1997); 26 to 86% females, (Verne et al. 2012). This is due to the presence of diverse sex-determining factors (Cordaux et al. 2011), like the feminizing endosymbiont *Wolbachia* (Bouchon et al. 1998) and nuclear f element (Leclercq et al. 2016), as well as the masculinizing M gene (Rigaud and Juchault 1993). This suggests that the intensity of sexual selection acting upon males also varies as a function of the prevalence of these factors.

Acknowledgements

We thank Alexandra Lafitte for technical assistance with live animals. This work has been funded by the 2015-2020 State-Region Planning Contracts (CPER), the European Regional Development Fund (FEDER), the partnership arrangements in ecology and the environment (DIPEE) and intramural funds from the Centre National de la Recherche Scientifique, and the University of Poitiers.

References

Adams EM, Jones AG, Arnold SJ (2005) Multiple paternity in a natural population of a salamander with long-term sperm storage. Mol Ecol 14:1803–1810. https://doi.org/10.1111/j.1365-294X.2005.02539.x

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164. https://doi.org/10.1006/anbe.2000.1446

Avise JC, Tatarenkov A, Liu J-X (2011) Multiple mating and clutch size in invertebrate brooders versus pregnant vertebrates. Proc Natl Acad Sci 108:11512–11517. https://doi.org/10.1073/pnas.1109216108

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349–368. https://doi.org/10.1038/hdy.1948.21

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R Package Version 11-7

Bech N, Depeux C, Durand S, et al (2017) When GIS zooms in: spatio-genetic maps of multipaternity in *Armadillidium vulgare*. Genetica 1–10. https://doi.org/10.1007/s10709-017-9992-8

Bouchon D, Rigaud T, Juchault P (1998) Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. Proc R Soc B-Biol Sci 265:1081–1090

Chapman T, Liddle LF, Kalb JM, et al (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory-gland products. Nature 373:241–244. https://doi.org/10.1038/373241a0

Coetzer WG, Downs CT, Perrin MR, Willows-Munro S (2017) Testing of microsatellite multiplexes for individual identification of Cape Parrots (*Poicephalus robustus*): paternity testing and monitoring trade. Peerj 5:e2900. https://doi.org/10.7717/peerj.2900

Coleman SW, Jones AG (2011) Patterns of multiple paternity and maternity in fishes. Biol J Linn Soc 103:735–760. https://doi.org/10.1111/j.1095-8312.2011.01673.x

Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53:1259–1267. https://doi.org/10.2307/2640828

Cordaux R, Bouchon D, Greve P (2011) The impact of endosymbionts on the evolution of host sex-determination mechanisms. Trends Genet 27:332–341. https://doi.org/10.1016/j.tig.2011.05.002

Coulon A (2010) genhet: an easy-to-use R function to estimate individual heterozygosity. Mol Ecol Resour 10:167–169. https://doi.org/10.1111/j.1755-0998.2009.02731.x

Dinno A (2016) Dunn's Test of Multiple Comparisons Using Rank Sums. R Package Version 132 Httpscranr-Proj

Durand S, Beauché F, Richard F-J, Beltran-Bech S (2015) How do females' genetic characteristics influence male mate preference in the terrestrial isopod *Armadillidium vulgare*? Ethology 121:1122–1130. https://doi.org/10.1111/eth.12429

322 Durand S, Cohas A, Braquart-Varnier C, Beltran-Bech S (2017) Paternity success depends on male
323 genetic characteristics in the terrestrial isopod *Armadillidium vulgare*. Behav Ecol Sociobiol
324 71:90. https://doi.org/10.1007/s00265-017-2317-1

325 Fortin M, Meunier J, Laverré T, et al (2019) Joint effects of group sex-ratio and *Wolbachia* infection
326 on female reproductive success in the terrestrial isopod *Armadillidium vulgare*. BMC Evol
327 Biol 19:65. https://doi.org/10.1186/s12862-019-1391-6

328 Fox J, Weisberg S, Adler D, et al (2017) car: Companion to Applied Regression. R package

329 Giraud I, Valette V, Bech N, et al (2013) Isolation and characterization of microsatellite loci for the
330 isopod crustacean *Armadillidium vulgare* and transferability in terrestrial isopods. Plos One
331 8:e76639. https://doi.org/10.1371/journal.pone.0076639

332 Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. J Hered 86:485–
333 486

334 Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific
335 variation and adaptive function. Mol Ecol 11:2195–2212. https://doi.org/10.1046/j.1365-
336 294X.2002.01613.x

337 Howard HW (1943) Length of life of sperms in the woodlouse *Armadillidium vulgare* Latr. Nature
338 152:331–331. https://doi.org/10.1038/152331c0

339 Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol
340 Rev 75:21–64. https://doi.org/10.1017/s0006323199005423

341 Jossart Q, Wattier RA, Kastally C, et al (2014) Genetic Evidence Confirms Polygamous Mating
342 System in a Crustacean Parasite with Multiple Hosts. PLOS ONE 9:e90680.
343 https://doi.org/10.1371/journal.pone.0090680

344 Kocher TD, Thomas WK, Meyer A, et al (1989) Dynamics of mitochondrial-DNA evolution in
345 animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A
346 86:6196–6200. https://doi.org/10.1073/pnas.86.16.6196

347 Krebs JR, Davies NB (1997) The evolution of behavioural ecology. In: Behavioral Ecology: An
348 Evolutionary Approach. Wiley

349 Leclercq Sb, Theze J, Chebbi MA, et al (2016) Birth of a W sex chromosome by horizontal transfer of
350 *Wolbachia* bacterial symbiont genome. Proc Natl Acad Sci U S A 113:15036–15041.
351 https://doi.org/10.1073/pnas.1608979113

352 Lefebvre F (2002) Stratégies de reproduction chez les crustacés isopodes terrestres. PhD dissertation,
353 Université de Poitiers

354 Lefebvre F, Limousin M, Caubet Y (2000) Sexual dimorphism in the antennae of terrestrial isopods: a
355 result of male contests or scramble competition? Can J Zool 78:1987–1993.
356 https://doi.org/10.1139/z00-128

357 Moreau J, Bertin A, Caubet Y, Rigaud T (2001) Sexual selection in an isopod with *Wolbachia*-induced
358 sex reversal: males prefer real females. J Evol Biol 14:388–394.
359 https://doi.org/10.1046/j.1420-9101.2001.00292.x

360 Moreau J, Seguin S, Caubet Y, Rigaud T (2002) Female remating and sperm competition patterns in a
361 terrestrial crustacean. Anim Behav 64:569–577. https://doi.org/10.1006/anbe.2002.4000

362 Pomiankowski A (1987) The costs of choice in sexual selection. J Theor Biol 128:195–218.
363 https://doi.org/10.1016/s0022-5193(87)80169-8

364 R Development Core Team (2013) R: A language and environment for statistical computing.

365 Reynolds JD (1996) Animal breeding systems. Trends Ecol Evol 11:A68–A72

366 Rigaud T, Juchault P (1993) Conflict between feminizing sex-ratio distorters and an autosomal
367 masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. Genetics 133:247–
368 252

369 Rigaud T, Juchault P, Mocquard JP (1997) The evolution of sex determination in isopod crustaceans.
370 Bioessays 19:409–416. https://doi.org/10.1002/bies.950190508

371 Rigaud T, Moreau M (2004) A cost of *Wolbachia*-induced sex reversal and female-biased sex ratios:
372 decrease in female fertility after sperm depletion in a terrestrial isopod. Proc R Soc B-Biol Sci
373 271:1941–1946. https://doi.org/10.1098/rspb.2004.2804

374 Rowe L (1994) The costs of mating and mate choice in water striders. Anim Behav 48:1049–1056.
375 https://doi.org/10.1006/anbe.1994.1338

376 Simmons LW, Beveridge M, Kennington WJ (2007) Polyandry in the wild: temporal changes in
377 female mating frequency and sperm competition intensity in natural populations of the
378 tettigoniid *Requena verticalis*. Mol Ecol 16:4613–4623. https://doi.org/10.1111/j.1365-
379 294X.2007.03528.x

380 Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. Genetics 137:1147–
381 1155

382 Surbida KL, Wright JC (2001) Embryo tolerance and maternal control of the marsupial environment
383 in *Armadillidium vulgare* (Isopoda : Oniscidea). Physiol Biochem Zool 74:894–906.
384 https://doi.org/10.1086/324474

385 Suzuki S, Ziegler A (2005) Structural investigation of the female genitalia and sperm-storage sites in
386 the terrestrial Isopod *Armadillidium vulgare* (Crustacea, Isopoda). Arthropod Struct Dev
387 34:441–454. https://doi.org/10.1016/j.asd.2005.06.002

388 Thrall PH, Antonovics J, Dobson AP (2000) Sexually transmitted diseases in polygynous mating
389 systems: prevalence and impact on reproductive success. Proc R Soc B-Biol Sci 267:1555–
390 1563

391 Uller T, Olsson M (2008) Multiple paternity in reptiles: patterns and processes. Mol Ecol 17:2566–
392 2580. https://doi.org/10.1111/j.1365-294X.2008.03772.x

393 Valette V, Durand S, Bech N, et al (2017) Multiple paternity in a wild population of *Armadillidium*
394 *vulgare*: influence of infection with *Wolbachia*? J Evol Biol 30:235–243.
395 https://doi.org/10.1111/jeb.13009

396 Vandel A (1962) Faune de France 66, Isopodes Terrestres, Paul Lechevalier. Paris

397 Verne S, Johnson M, Bouchon D, Grandjean F (2012) Effects of parasitic sex-ratio distorters on host
398 genetic structure in the *Armadillidium vulgare-Wolbachia* association. J Evol Biol 25:264–
399 276. https://doi.org/10.1111/j.1420-9101.2011.02413.x

400 Verne S, Moreau J, Caubet Y, et al (2007) Male mating success during parturial intermoults in the
401 terrestrial isopod *Armadillidium vulgare* revealed by the use of a microsatellite locus. J
402 Crustac Biol 27:217–219. https://doi.org/10.1651/s-2752.1

403 Verne S, Puillandre N, Brunet G, et al (2006) Characterization of polymorphic microsatellite loci in
404 the terrestrial isopod *Armadillidium vulgare*. Mol Ecol Notes 6:328–330.
405 https://doi.org/10.1111/j.1471-8286.2006.01220.x

406 Wacker S, Larsen BM, Jakobsen P, Karlsson S (2018) High levels of multiple paternity in a spermcast
407 mating freshwater mussel. Ecol Evol 8:8126–8134. https://doi.org/10.1002/ece3.4201

408 Walker D, Porter BA, Avise JC (2002) Genetic parentage assessment in the crayfish *Orconectes*
409 *placidus*, a high-fecundity invertebrate with extended maternal brood care. Mol Ecol 11:2115–
410 2122. https://doi.org/10.1046/j.1365-294X.2002.01609.x

411 Yasui Y (1998) The "genetic benefits" of female multiple mating reconsidered. Trends Ecol Evol
412 13:246–250. https://doi.org/10.1016/s0169-5347(98)01383-4

413 Yue GH, Chang A (2010) Molecular evidence for high frequency of multiple paternity in a freshwater
414 shrimp species *Caridina ensifera*. Plos One 5:. https://doi.org/10.1371/journal.pone.0012721

415 Ziegler A, Suzuki S (2011) Sperm storage, sperm translocation and genitalia formation in females of
416 the terrestrial isopod *Armadillidium vulgare* (Crustacea, Peracarida, Isopoda). Arthropod
417 Struct Dev 40:64–76. https://doi.org/10.1016/j.asd.2010.07.003

418