Exp Appl Acarol (2017) 71:63–70
DOI 10.1007/s10493-016-0098-2

A facultative ectoparasite attains higher reproductive success as a parasite than its free-living conspecifics

Lien T. Luong[1] · **Dewmi Subasinghe**[1]

Received: 15 July 2016 / Accepted: 3 November 2016 / Published online: 17 November 2016

Abstract Comparative and phylogenetic studies reveal that parasites evolved from free-living ancestors, but the underlying processes and mechanisms are not well understood. In order for selection to favor the evolution of parasitism, there must be pre-existing adaptations for host exploitation and a fitness advantage favoring the transition to parasitism. Here, we experimentally investigate the differential reproductive success of a facultative parasite, *Macrocheles subbadius*. Lifetime reproductive success was higher among female mites that fed on fruit fly hemolymph as parasites compared to free-living mites. The per capita lifetime fecundity for parasitic females was 2.4 times that of free-living females. The offspring sex ratio also differed between the two modes of life, with parasitic mites producing more sons and hence a relatively weaker female-biased sex ratio (0.61) than mites that were free-living (0.94). These results suggest that parasitic female mites allocate more resources to egg production, and were limited by sperm rather than food, as was the case with free-living mites. The higher lifetime reproductive success of mites that opportunistically feed on host hemolymph provides the fitness advantage necessary for the evolution of parasitism.

Keywords Drosophila · Evolution of parasitism · Facultative parasitism · Lifetime reproductive success · Fecundity · *Macrocheles* · Sex-ratio

Introduction

Parasites evolved from free-living ancestors, but little is known about the mechanisms and processes involved, on an ecological or evolutionary time scale (Poulin 2007). Phoresy may serve as an important evolutionary stepping-stone towards parasitism, especially

---

✉ Lien T. Luong
lluong@ualberta.ca

[1] Department of Biological Sciences, University of Alberta, CW405 Biological Sciences Bldg., Edmonton, AB T6G 2E9, Canada

among mites and nematodes (Athias-Binche and Morand 1991; Houck and OConnor 1991). Phoresy is a phenomenon in which one animal attaches to the body of a larger animal for the purpose of dispersal (Athias-Binche 1993; Houck and OConnor 1991). Typically the phoront is quiescent and suspends feeding and development until it arrives in a new habitat and detaches (Athias-Binche and Morand 1991; Houck and OConnor 1991). One of the criteria for delineating an organism as phoretic versus parasitic is that the latter obtains nutritional benefits from the host to the detriment of the host (Houck 1994; Walter and Proctor 2013). In addition to pre-existing adaptations for survival and feeding on a host, there must exist a fitness advantage favoring the evolution of parasitism, i.e., the reproductive success of a parasite must be greater than that of a conspecific that does not feed on a host. In this scenario, phoretic forms would eventually become specialized to exploit host tissue/hemolymph, becoming parasitic by definition (Anderson 1984; Walter and Proctor 2013). The assumption is that the fitness advantages of a parasitic life-style must be greater than phoresy alone and outweigh the potential costs of a parasitic life-style, such as adaptation to chemicals in the hemolymph and overcoming host immune defenses (Houck 1994). To our knowledge, no studies to date have experimentally investigated the fitness benefits of parasitism in a taxon where a continuum ranging from free-living to parasitism exists.

Facultative parasitism may also be an important evolutionary step towards obligate parasitism. For example, isopods of the family Cymothidae are obligate ectoparasites of fish but had ancestors that were facultative parasites (Brusca 1981). Facultative parasites are capable of parasitizing a host but retain the ability to reproduce and develop in a free-living environment. *Machrocheles subbadius* Berlese (Acari, Macrochelidae) is a facultative ectoparasitic mite that lives in rotting cacti and feeds on nematodes and other small invertebrates during its free-living stages, mature females attach to adult fruit flies, including *Drosophila nigrospiracula* Patterson and Wheeler when the microhabitat deteriorates. While other members of this genus are purely phoretic, *M. subbadius* is known to extract and consume hemolymph from the host, with deleterious effects on host fitness (Polak 1996, 1998). The *M. subbadius*—fly association is an ideal system for investigating the evolution of parasitism because the mites can utilize the fly hosts for dispersal and for nutrition; hence the relationship is both phoretic and facultatively parasitic. We hypothesize that in order for parasitism to evolve from a phoretic ancestor, there must exist a clear fitness advantage to extracting host resources. Specifically, we predict that the *M. subbadius* mites that have access to hosts will have higher fitness in terms of lifetime fecundity compared to their free-living conspecifics.

Materials and methods

Flies and mites

Both flies and mites were originally collected from necrotic saguaro cacti, *Carnegiea gigantean* Britton & Rose (Cactaceae) in the Sonoran Desert, Arizona, USA in 2015. Mites were cultured and maintained in mass culture as described by Luong et al. (2015) under standard laboratory conditions (12 h light, 25 °C: 12 h dark, 24 °C, 70% RH). The mite media consists of moist wheat bran, wood shavings, and Rhabditida bacteriophagic nematodes (source, M. Polak, University of Cincinnati, OH, USA) as food. Flies were cultured in media containing instant potato flakes, *Drosophila* medium (Formula 4–24

Instant *Drosophila* Medium, Carolina Biological Supply Company, Burlington, NC, USA), active yeast, and a small amount of autoclaved necrotic cactus. Fly cultures were maintained under similar conditions as the mites but in a separate incubator.

Experimental protocol

Two experimental groups were established, a ‘parasitic’ group of mites that were allowed to feed on fly hemolymph and a control or ‘free-living’ group with no access to fly hosts. Thirty female deutonymphs and 30 adult males were taken from the mass culture. They were equally distributed between two 100 ml specimen containers with 50 ml of culture media (15 females and 15 males/container). The containers were then left undisturbed for two days to allow the females to mate and mature. For the parasitic group, two mites were transferred to an infection chamber with a single female *D. nigrospiracula* fly. Infection chambers were constructed from modified 200 μL pipette tips (truncated and stoppered with cotton) to produce a restricted environment for the mite to attach to the fly. The infection chambers were then covered and left undisturbed for 2 h to allow the mites to attach. Free-living mites were placed in similar tubes but without access to a fly. This experimental protocol was replicated to achieve a total of $n = 23$ free-living mites and $n = 21$ parasitic mites.

At the end of the 2-h attachment period, the infection chambers were examined. Mites that had access to a fly but failed to attach were discarded. Mites that were still attached to a fly after 24 h were individually transferred to 100 ml specimen containers with 50 ml of media. Each female was transferred to a new container with fresh media every 3–4 days until she died. Longevity was measured as the number of days alive from deutonymph stage to death. All containers with progeny were maintained for an additional 3–5 days to allow the mites to mature so that offspring sex could be determined. The media in the container was then preserved in 70% ethanol. Since only adult progeny were counted (see below), lifetime reproductive success is measured as the total number of offspring that survive to adulthood.

Washing techniques

Method 1

Preserved media containing mites were washed twice through a coarse sieve (1 mm mesh) onto a fine sieve (100 μm mesh). Using a wash bottle, the debris collected on the fine sieve were transferred to a 15-cm Petri dish. The Petri dish was examined under a dissecting microscope, and the mites present were counted. This method was used to count the offspring of 10 ‘free-living’ female mites and 7 ‘parasitic’ females.

Method 2

Preserved media were transferred to a 140 ml snap cap vial. The rest of the vial was filled with water and sealed with Parafilm M®. The container was gently agitated to mix the contents and then set aside to allow the sediments to settle. Once the sediment settled, the supernatant (containing suspended mites) was poured into a 15-cm Petri dish and examined under a dissecting microscope. This extraction process was repeated 3 more times per container to ensure a complete count. This method was applied to assess offspring count for 13 ‘free-living’ females and 14 ‘parasitic’ female mites. We switched to Method 2 because it was less time consuming. These two methods yielded statistically comparable recoveries (GLM, deviance = 5.81, $p = 0.24$).

Data analysis

Data were analysed using the R statistical program (R Core Team 2015). Generalized linear models (GLM) were used to test for the effect of treatment, days alive, and washing method on offspring production. A factor was retained if the model comparison showed increased deviance in the reduced model without the variable ($p \leq 0.05$, Chi square statistic). A quasi-Poisson model was used to fit the fecundity data due to over-dispersion. Longevity (i.e., number of days alive) was modeled using a gamma error distribution, with treatment and washing method as independent factors.

Results

More female mites (76%; 16/21) from the parasitic group reproduced compared to females (44%; 10/23) in the free-living group; this difference was not statistically significant ($\chi^2 = 3.60$, $p = 0.06$). The females that fed on fly hemolymph produced significantly more offspring over their lifetime than free-living mites (deviance = −18.5, $p = 0.045$; Fig. 1). The average per capita lifetime fecundity for the parasitic mites (3.76 ± 0.77 SE) was more than double that of the free-living mites (1.57 ± 0.55 SE). The mean number of female offspring was slightly higher among parasitic mites compared to the free-living mites, but the difference was not statistically significant (deviance = −3.75, $p = 0.34$). As a group, parasitic mites produced 48 females whereas free-living mites produced a total of 34 females. The mean number of male offspring was statistically higher among mites that fed on a fly host (deviance = 32.7, $p = 0.0003$, Fig. 1). In the free-living group, only 2 males were produced whereas the parasitic group as a whole yielded 31 male offspring. The female-biased sex ratio was much more prominent in the free-living group (proportion of females = 0.94) compared to the parasitic group (prop. female = 0.61; $\chi^2 = 3.12$, $p = 0.0005$).

Longevity was also an important factor in the fecundity model (deviance = −19.5, $p = 0.04$; Fig. 2), but the interaction between treatment and longevity was not significant ($p > 0.05$). The mean number of days alive for free-living (6.65 ± 3.74 SD days) and parasitic (7.48 ± 2.54 SD days) mites was comparable (deviance = −0.15, $p = 0.42$).

Discussion

As predicted by the fitness advantage hypothesis, lifetime fecundity was significantly higher among parasitic mites compared to free-living conspecifics. From a mechanistic standpoint, fly hemolymph likely enables the parasitic mites to allocate more resources towards egg production. For instance, female mosquitoes blood feed on vertebrate hosts to obtain protein and other nutrients for egg production (Dodd and Burgess 1995; Roitberg and Gordon 2005). Likewise, tick fecundity is a function of the size of the blood meal (Sonenshine 1991). All else being equal, the increase in relative fitness obtained from *M. subbadius* opportunistically attaching to and feeding on host hemolymph provides the

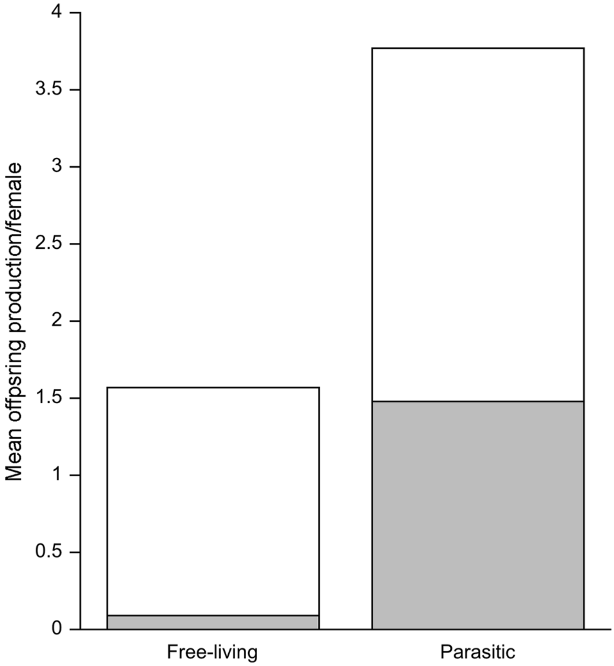

Fig. 1 Average number of male (*grey bar*) and female (*white bar*) offspring produced per female mite among free-living (n = 23) versus parasitic (n = 21) *M. subbadius*

advantage necessary for the evolution of parasitism, i.e., as an evolutionary a stepping-stone.

In this study, we did not track the lifetime fecundity of mites that were exposed to flies but did not attach. It is possible that the ‘parasitic’ group represents a subset of mites that happen to be more inclined to attach (fertilized and/or gravid with mature eggs) or simply more vigorous and hence able to overcome host defenses and successfully attach. However, the proportion of female mites that produced offspring was not statistically different between the parasitic and control group. Also, mites from both groups were reared under similar food and mating conditions (note: virgin, unmated females are just as likely to attach, pers. observations). Hence the observed difference in fecundity between the two groups is unlikely due to variation in mating status. Differences in mite ‘vigor’ are also unlikely to be important given that the infection chambers used in this study strongly restricted fly movement, the primary line of defense against mite attack (Luong et al. 2007). The conclusion that mites attaching to flies gain a nutritional benefit, which translates into higher lifetime fecundity, is further supported by the biased sex ratio among parasitic females.

The reproductive mode of *M. subbadius* is arrhenotoky, which provides a mechanism by which females are able to control the sex ratio of their offspring (Hamilton 1967). A female-biased sex-ratio is common in haplodiploid, arrhenotokous systems (Hamilton 1967; Wrensch and Ebbert 1993). Young et al. (1986) showed that female spider mites produced fewer sons when reared on poor quality food compared to high quality food. We hypothesize that female mites with access to flies and potentially higher quality food (i.e., fly hemolymph) responded with increased egg production but remained sperm limited.

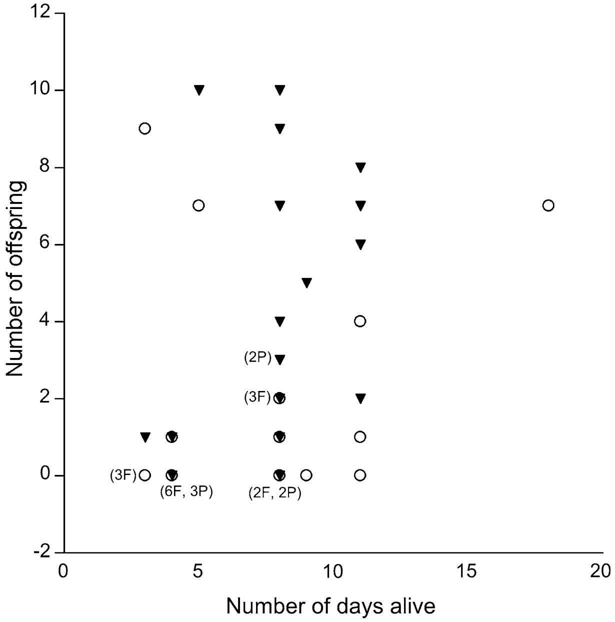

Fig. 2 Relationship between longevity (number of days alive from deutonymph stage to death) and lifetime fecundity of free-living (F, *dots*) and parasitic (P, *triangles*) females of *M. subbadius*. *Numbers in parentheses* indicate overlapping data points for replicate mites

This may explain the greater production of sons among mites that fed on flies compared to mites that were primarily food limited. Sex allocation theory predicts that females should adjust the sex ratio of their offspring when their overall fitness is higher for one sex over the other (Trivers and Willard 1973). Indeed, the ideal sex ratio likely depends on the degree of local mate competition, stage of colonization, quality of food, and density in a given patch (Wrensch 1993).

Few studies have examined the relationship between the evolution of parasitism and fecundity. Poulin (1995) compared parasitic taxa of copepods and isopods with free-living sister taxa, and found that parasitic taxa were more fecund than their closest free-living relatives. However, this pattern does not appear to be the case among parasitic and free-living Platyhelminthes (Trouve et al. 1998). While comparing sister taxa can account for some confounding factors associated with phylogenetic history, facultative parasites offer an opportunity to examine the life-history traits of free-living versus parasitic strategies within a single species. Such studies are rare, but one tantalizing case comes from a facultative nematode parasite of siricid woodwasps that feed on fungi during the free-living phase. Bedding (1972) noted that *Deladenus sircidicola* nematodes cultured on fungi produced in "excess of 1000 eggs" per female, whereas parasitic females produced an average of 2000 offspring.

Phoresy is commonly cited as a possible evolutionary step between a free-living and parasitic lifestyle, particularly among nematodes and mites (Athias-Binche and Morand 1991; Poulin 2007; Walter and Proctor 2013). Houck (1994) suggested that deutonymphs of *Hemisarcoptes cooremani* Thomas (Hemisarcoptidae) are in the process of evolving from phoresy to parasitism. The deutonymphs appear to extract and feed on host hemolymph (Houck and Cohen 1995). Houck (1994) proposed a series of hypothetical steps for the evolutionary transition from phoresy to parasitism in Astigmata. Phoretic forms possess the pre-adaptations for host exploitation, e.g., extended association with the host and specialized structures for host seeking and attachment. The next step would involve adaptation to host defenses and utilization of hemolymph as a nutritional source. Of course, the evolutionary transition to parasitism will not proceed without the presence of fitness benefits; the reproductive success of a parasite must be higher than that of a conspecific that does not feed on a host. Our study provides empirical support for this last and perhaps most important criterion. Hence, *M. subbadius* may represent an evolutionary stepping-stone towards parasitism. Alternatively, polymorphism in resource exploitation strategies may be maintained in natural populations by various evolutionary constraints such as genetic and physiological trade-offs associated with increased reliance on hosts to complete its life cycle. Our findings shed light on the ecological conditions necessary for the evolutionary transition to parasitism.

Acknowledgements We would like to thank H. Proctor for providing valuable discussions and A. Shostak for constructive comments on the manuscript. We also thank M. Farrell for maintaining the fly cultures. LTL was supported by the Natural Sciences and Engineering Research Council of Canada.

References

Anderson RC (1984) The origins of zooparasitic nematodes. Can J Zool-Rev Can Zool 62:317–328

Athias-Binche F (1993) Dispersal in varying environments: the case of phoretic uropodid mites. Can J Zool-Rev Can Zool 71:1793–1798

Athias-Binche F, Morand S (1991) From phoresy to parasitism: the example of mites and nematodes. Res Rev Parasitol 53:73–79

Bedding RA (1972) Biology of *Deladenus siricidicola* (Neotylenchidae) an entomophagous-mycetophagous nematode parasitic in Siricid woodwasps. Nematologica 18:482–493

Brusca RC (1981) A monograph on the Isopoda, Cymothoidae (Crustacea) of the Eastern Pacific. Zool J Linn Soc 73:117–199. doi:10.1111/j.1096-3642.1981.tb01592.x

Dodd CS, Burgess NR (1995) Why do insects bite? A review of blood sucking behaviour. J R Army Med Corps 141:151–156

Hamilton WD (1967) Extraordinary sex ratios. Science 156:477–488. doi:10.1126/science.156.3774.477

Houck MA (1994) Adaptation and transition in parasitism from commensalism: a phoretic model. In: Houck MA (ed) Mites: ecological and evolutionary analyses of life-history patterns. Chapman and Hall, New York, pp 252–281

Houck MA, Cohen AC (1995) The potential role of phoresy in the evolution of parasitism: radiolabelling (tritium) evidence from an astigmatid mite. Exp Appl Acarol 19:677–694. doi:10.1007/bf00052079

Houck MA, OConnor BM (1991) Ecological and evolutionary significance of phoresy in the Astigmata. Annu Rev Entomol 36:611–636

Luong LT, Heath BD, Polak M (2007) Host inbreeding increases susceptibility to ectoparasitism. J Evol Biol 20:79–86. doi:10.1111/j.1420-9101.2006.01226.x

Luong LT, Penoni LR, Horn CJ, Polak M (2015) Physical and physiological costs of ectoparasitic mites on host flight endurance. Ecol Entomol 40:518–524. doi:10.1111/een.12218

Polak M (1996) Ectoparasitic effects on host survival and reproduction: the *Drosophila-Macrocheles* association. Ecology 77:1379–1389

Polak M (1998) Effects of ectoparasitism on host condition in the *Drosophila-Macrocheles* system. Ecology 79:1807–1817

Poulin R (1995) Clutch size and egg size in free-living and parasitic copepods—a comparative analysis. Evolution 49:325–336. doi:10.2307/2410343

Poulin R (2007) Evolutionary Ecology of Parasites. Princeton University Press, Princeton

Roitberg BD, Gordon I (2005) Does the *Anopheles* blood meal-fecundity curve, curve? J Vector Ecol 30:83–86

Sonenshine DE (1991) Biology of ticks, vol part 1. Oxford University Press, New York

Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. Science 4068:90–92

Trouve S, Sasal P, Jourdane J, Renaud F, Morand S (1998) The evolution of life history traits in parasitic and free-living platyhelminthes: a new perspective. Oecologia 115:370–378. doi:10.1007/s004420050530

Walter DE, Proctor HC (2013) Mites: ecology, evolution, and behaviour. Oxford University Press, New York

Wrensch DL (1993) Evolutionary flexibility through haploid males or how chance favors the prepared genome. In: Wrensch DL, Ebbert MA (eds) Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York

Wrensch DL, Ebbert MA (1993) Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York

Young SSY, Wrensch DL, Kongchuensin M (1986) Control of sex-ratio by female spider mites. Entomol Exp Appl 40:53–60