The Science of Nature (2019) 106: 23
https://doi.org/10.1007/s00114-019-1616-y

ORIGINAL PAPER

First come, first served: precopulatory mate-guarding behavior and male–male contests by a hymenopteran saproxylic parasitoid

Kazumu Kuramitsu[1] · Thitaree Yooboon[2,3] · Morihiko Tomatsuri[4] · Hideo Yamada[5] · Tomoyuki Yokoi[1]

Received: 11 December 2018 / Revised: 28 March 2019 / Accepted: 5 April 2019 / Published online: 8 May 2019

Abstract

Precopulatory mate-guarding behavior is a common strategy that maximizes male reproductive success when female receptivity to copulation is low. This behavior has been demonstrated in vertebrates, aquatic crustaceans, terrestrial isopods, and some species of insects, but there is very little available information about hymenopteran insects. A few studies have clarified the factor that determines the outcome of a contest between a guarding male and an invader male. We investigated the male–male contest and mating behavior of a saproxylic parasitoid wasp, *Ibalia japonica* (Hymenoptera: Cynipoidea: Ibaliidae) using field observations in Japan. These observations indicated that *I. japonica* males show precopulatory mate-guarding behavior and that four types of male–male contests occur on the *Magnolia liliiflora* (Magnoliales: Magnoliaceae) tree that virgin females emerge from. We show that the arrival order of *I. japonica* males that found the future emergence point of a female was key factor that allowed males to secure virgin females.

Keywords *Ibalia japonica* · Ibaliidae · Intra-sex competition · Mating behavior · Siricidae · Woodwasp

Introduction

Mate-securing is critical for sexual reproduction in animals. Precopulatory mate-guarding behavior is a common strategy used by males to maximize reproductive success when females show low receptivity to copulation (Jormalainen 1998), and the strategy has been demonstrated in both vertebrates (Low 2006; Schubert et al. 2009; Brattli et al. 2018) and invertebrates (Jormalainen 1998; Wada et al. 1999; Iltis et al. 2017). In some species, male–male contests for a female occur between a guarding male and a newly arrived male. Male–male contests and precopulatory mate-guarding behavior by invertebrates are well documented in aquatic crustaceans (Wada et al. 1997, 1999; Jormalainen 1998; Murai and Backwell 2005), and some studies on insects, such as Odonata (Miller et al. 1984), Coleoptera (Arakaki et al. 2004; Chaudhary et al. 2017), and Lepidoptera (Bennett et al. 2012; Jarrige et al. 2016), are available. However, only a few studies have focused on mate-guarding behavior by hymenopteran insects (Longair 2004; Beani et al. 2014), and there is no available information about hymenopteran parasitoids, although many parasitoid species are known to have male–male contests to access virgin females (Godfray 1994). Furthermore, a few studies have demonstrated that there are several factors that can determine the outcome of contests between a guarding male and an invader male in mate-guarding contests, such as body size or the size of specific body parts (Brockerhoff and McLay 2005; Walker and Holwell 2018).

We investigated the precopulatory male–male contests and mating behavior of newly emerged *Ibalia japonica* (Hymenoptera: Cynipoidea: Ibaliidae), which is a primary

Communicated by: Lars Koerner

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00114-019-1616-y) contains supplementary material, which is available to authorized users.

✉ Kazumu Kuramitsu
kuramitsu.kazumu.ws@alumni.tsukuba.ac.jp

[1] Laboratory of Conservation Ecology, Faculty of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[2] Laboratory of Applied Entomology and Zoology, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[3] Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

[4] Shimoda Marine Research Center, University of Tsukuba, 5-10-1, Shimoda, Shizuoka 415-0025, Japan

[5] Tsukuba Experimental Forest, Mountain Science Center, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan

solitary, koinobiont, endoparasitoid of the woodwasp *Tremex apicalis* (Hymenoptera: Siricidae) (Nordlander and Liu 1994; Choi et al. 2013; Kuramitsu et al. 2016; Kuramitsu et al. 2019). *Ibalia* spp. larvae parasitize eggs and larvae of wood-boring woodwasps, which are forest pests. Adult *Ibalia* spp. emerge from the host-infested wood after chewing an exit hole through the wood and bark (Nordlander and Liu 1994). *Ibalia drewseni* adults are known to mate after emergence from host-infested wood (Spradbery 1974), but the mating process for ibaliid species is largely unknown. Females of most parasitoid (Godfray 1994) and Cynipoidea species (Abe 1991) are known to mate only once in their life.

To clarify the determinants of the male–male contest, we investigated the arrival order of males to the female emergence point and body size effects on male–male contest outcomes. The principal aim of this study was (1) to determine whether the hymenopteran parasitoid, *I. japonica*, shows precopulatory mate-guarding behavior and (2) to clarify the determinant that is responsible for the male–male contest outcomes.

Materials and methods

Study site and study organisms

Field observations of *I. japonica* were carried out in the Tsukuba Experimental Forest (36° 07′ 10″ N; 140° 05′ 50″ E [DMS], ca. 25 m a.s.l.), University of Tsukuba, Ibaraki Prefecture, Japan. We identified one tree of *Magnolia liliiflora* (Magnoliales: Magnoliaceae) that had been infested by *T. apicalis* in 2017. This tree was approximately 12 m in height and 44 cm in diameter at breast height. To observe emergence and mating behavior of *I. japonica*, the infested parts of this tree were removed with a chainsaw on October 4, 2017 and the wood kept outside until May 2018. Behavior of males and females of *I. japonica* that emerged from the bolts of the infested wood was observed. In this study, we did not individually identify male wasps in the field. Therefore, we cannot deny the possibility that our data included some pseudoreplication.

Observation 1: descriptions of the *I. japonica* mating process and mate–mate contests

Field observation 1 lasted for 5 days from May 8 to May 15, 2018. We observed the woodwasp-infested *M. liliiflora* between 10:00 and 16:00 on each day. The behavior of both *I. japonica* males and females was recorded when we found newly emerging females on the wood. When a male mounts a female, we observed whether the female resists to copulation or not. Seven of the 34 female emergences were recorded by a video camera (Tough TG-5, Olympus Corporation, Tokyo, Japan or iPhone SE, Apple Inc., Cupertino, USA).

Observation 2: effects of arrival sequence and/or male body size on male–male contests

Observation 1 indicated that *I. japonica* males showed male–male contest behavior around the female emergence site (see below). To clarify the contributing factors that influence the outcome of their contests, we evaluated the effects of (1) the arrival sequence of the males at the potential female emergence point and (2) male body size.

The relationship between the order of arrival at the potential female emergence point and the outcome of the male–male contests was determined from videos that were taken during observation 1. Contest types and the outcome of the contests, such as the guarding male winning against an invader, were recorded. The competition was recorded as a draw when both the guarding male and the invader male left the female emergence point after a contest. Body size effects on the outcome of a male–male contests were determined by field observations that were carried out between May 15 and 20, 2018. When a male–male contest around a female emerging point was identified, we caught the losing male after their contest and measured its body length and the tibia length of a right foreleg with digital venire calipers (DN-150, Niigata Seiki Co., Ltd., Sanjo, Japan). The winning male was also caught after mating, and its body was measured in a similar way to the losing male. Losing males were released after mating of the female and the winning male. The winning male was also released after the measurement of its body size.

Finally, the successful mating rate of the first male (the male that found a newly emerging female first) was calculated based on information from observation 1.

Statistical analysis

All statistical analyses were performed using R v. 3.4.2 (R Core Team, 2015). Significance of winning percentage of wasps in each contest was analyzed using a binominal test.

Results

Observation 1: description of the mating process and the *I. japonica* male–male contests

We observed 34 emerging *I. japonica* females. A diagram of the normal mating sequence is shown in Fig. 1. A total of 94.1% mated immediately after emergence from the wood (Fig. 2; Online Resource 1). The male that stood by the female emergence point immediately mounted the female after her emergence. All of the guarding males ($n = 32$) touched

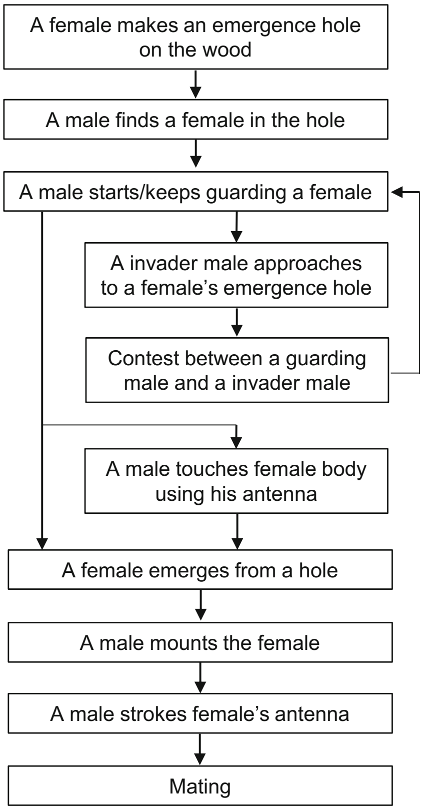

Fig. 1 A diagram of the normal mating sequence shown by *I. japonica*

females' body using their antenna during female emergence. Before and during mating, the males stroked the female antenna using their own antenna (Online Resource 1). Copulation duration was 58.8 ± 18.4 s (mean ± SD, $n = 7$). During copulation, other males tried to interrupt the mating pairs 1.57 ± 1.51 times (mean ± SD, $n = 7$) per pair but no invading males obtain females. Female resistance to first copulation was never observed, but mated females were not receptive to further courtship after first mating. Two females emerged when no male was waiting near their emergence hole, and these females flew away from the wood.

Based on the video analysis, total 203 male–male contests performed by a cumulative total of 176 male–male pairs were observed on the wood in advance of mating. When another male approached the emergence spot, a contest between the guarding male and invader male occurred. The male–male contests can be classified to four types, i.e., repelling (running at the invader with wings flapping and sounds; Online Resource 2), mounting (Fig. 3a; Online Resource 3), biting (biting invader's antenna; Fig. 3b; Online Resource 4), and foreleg spreading (Fig. 3c, d; Online Resource 5). In many cases, the guarding male raised his head and looked around the emergence spot, while the invader male approached the emergence spot with antennal searching on the wood. Therefore, it appears that the guarding male began to attack an invader before the invader noticed the guarding male. The guarding males guarded a hole for 9.14 ± 5.33 min (mean ± SD, $n = 7$) before a female came out. No females emerged from hole during male–male contests.

Observation 2: effects of the order in which males found the female and body size on male–male contests

A transition diagram for 176 male–male pairs, based on 203 male–male contests, was analyzed. Repelling behavior was a dominant contest type (65.3%) followed by mounting (17.0%), foreleg spreading (15.9%), and biting (1.7%), which was rarely observed. A total of 84.6% of the couple interactions were settled by a single contest type, and the others (15.4%) were

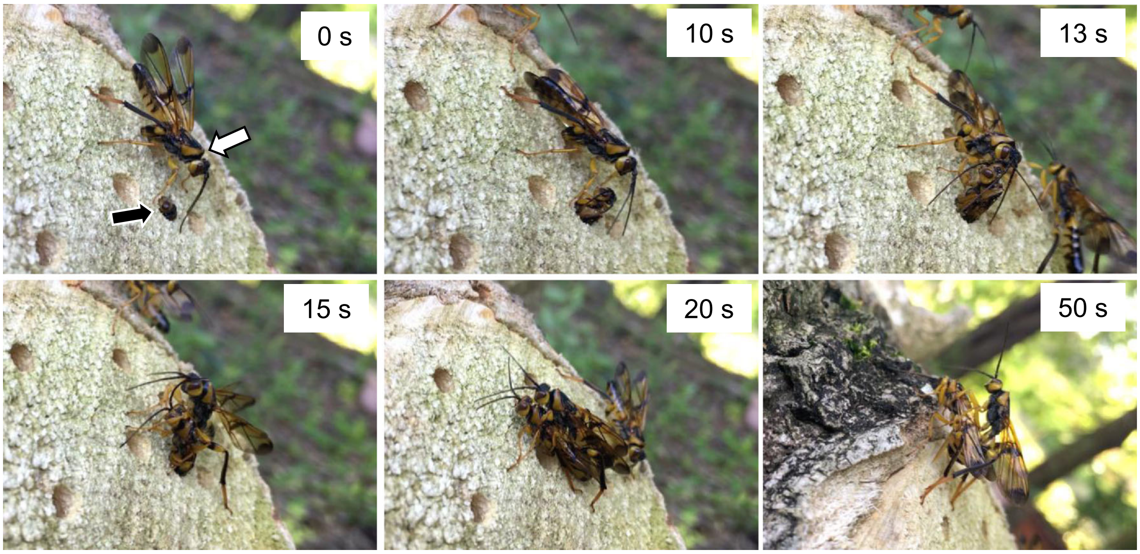

Fig. 2 Sequential images of female emergence and male mating behavior shown by *I. japonica* (Supplementary material 1). The black arrow indicates the female, and the white arrow indicates the male

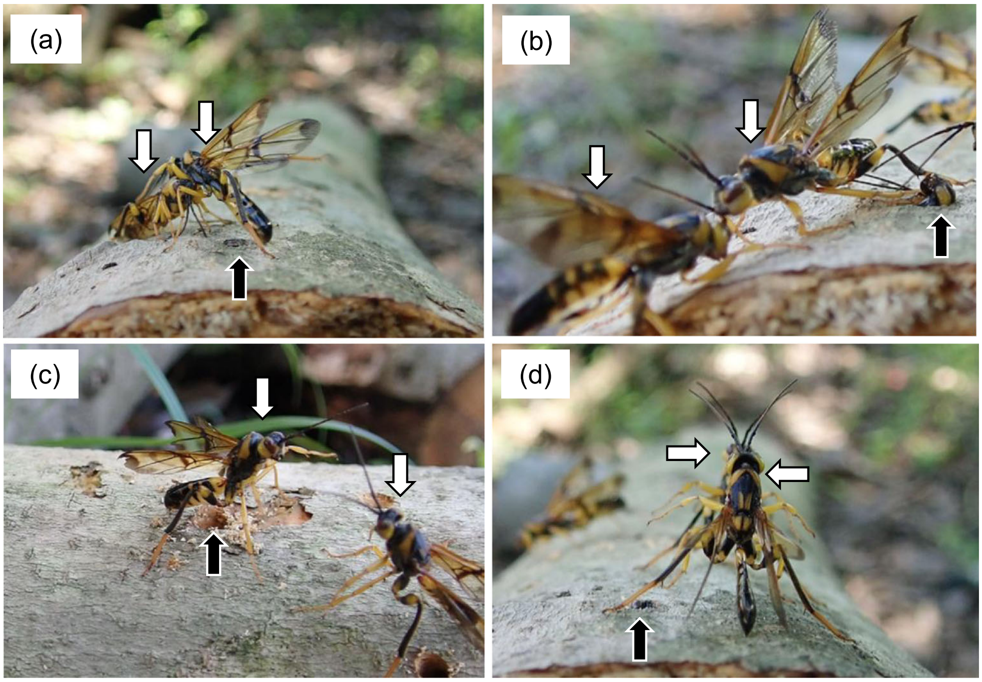

Fig. 3 *Ibalia japonica* male–male contest types. Mounting (**a**), biting (**b**), and fore leg spreading (**c**, **d**) behavior of *I. japonica* males. Black arrows indicate newly emerging females or emergence holes containing a newly emerging female, and white arrows indicate fighting males

settled by two contest types. No pairs performed three or more contest types in the same male–male contest (Fig. 4).

The percentage of wins by a guarding male was 95.0% in total and 82.1–100% for each contest type (Fig. 5). Guarding males showed a significantly higher winning percentage ($P < 0.01$, binomial test, $n = 202$) in a contest against an invader (Fig. 5).

Ibalia japonica males showed small variations in body size, i.e., body length was 18.7 ± 1.3 mm (mean ± SD, $n = 36$) and foreleg length was 2.7 ± 0.2 mm (mean ± SD, $n = 36$). Guarding males won significantly more contests (binomial test, $P < 0.01$) against both smaller invaders (81.8%, $n = 22$) and larger invaders (88.5%, $n = 26$). The percentage of females that mated with a first arrival male (87.5%) was significantly higher ($P < 0.001$, binomial test, $n = 32$) than with second or later males (12.5%).

Discussion

Ibalia japonica males awaited female emergence and mated immediately with virgin females (Fig. 2; Online Resource 1). Along with many other parasitoid species (Ruther 2013), *Ibalia japonica* may also release a female-derived courtship pheromone, which is detected by the males and allows them to find and mate with newly emerging females. Rhyssini parasitoid males (Hymenoptera: Ichneumonidae), which are another woodwasp parasitoids, also aggregate and wait around the female emergence points (Quicke 2014). This suggests that waiting for female emergence on the wood surface is a common strategy for saproxylic parasitoid males. *I. japonica* males stroked the female antenna during mating (Online Resource 1). The behavior seems to be a signal for the female to accept the male, and this occurs amongst other Cynipoidea

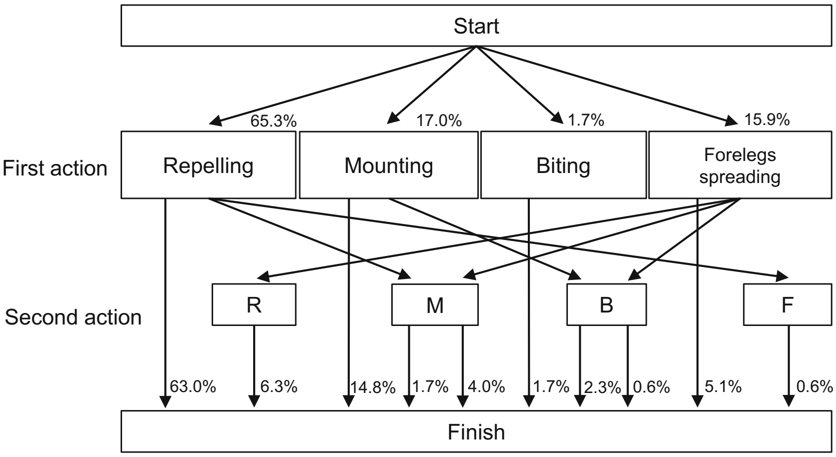

Fig. 4 Transition diagram for *I. japonica* male–male contests. Abbreviations for the second actions are as follows: R repelling, M mounting, B biting, F foreleg spreading ($n = 176$)

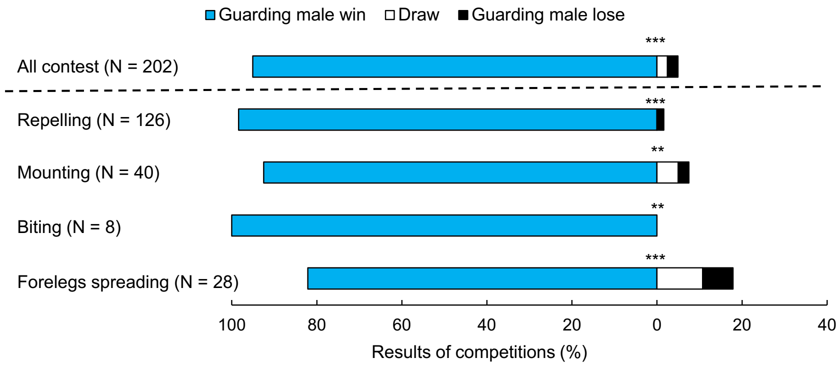

Fig. 5 Results of male–male contests for each contest type. Asterisks indicate a significant difference between "win" and "draw + lose": $^{**}P < 0.01$, $^{***}P < 0.001$ (binomial test)

(Isidoro et al. 1999) and Vespidae (Romani et al. 2005). No females emerged from a hole during male–male contests. Female wasps may detect male movement or sound in order to emerge safety.

There were contests between a guarding male and invaders (176 male–male pairs showed 203 contests). Winners of the contest stayed at the female emergence point and the losers left. We conclude that the purpose of the contests is to secure a virgin female. Contest styles were classified into four types. A few individuals used two contest styles although there was no characteristic combination (Fig. 4). Some parasitoid Chalcidoidea (Hymenoptera) species are also known to perform male–male contests to secure newly emerged females and they also use multiple contest styles (Wilson 1961; van den Assem et al. 1980). For example, Wilson (1961) showed that once an *Asolcus basali* (Hymenoptera: Scelionidae) male takes possession of a host egg mass from which female wasps will emerge, the male drives away all other males by attacking or biting. Other chalcidoid parasitoids also show mounting, repellent, or biting behavior during male–male contests (van den Assem et al. 1980; Godfray 1994). Taken together, the data suggest that these three behaviors are positively selected in several parasitoid taxa. In contrast, foreleg spreading has never been observed in other species. Therefore, that behavior might be unique to *I. japonica*.

Most guarding males won the contest regardless of contest type (Fig. 5) and showed a high winning percentage even when an invader was bigger than a guarding male. In many cases, guarding males launched the attack on invaders without being noticed. These data clearly indicate that guarding males make a preemptive attack against invaders, and it is often sufficient to settle the contests. Body size and/or the size of specific body parts are major factors that affect the outcome of male–male fights in many animal taxa (Christy 1983; Longair 2004; Brockerhoff and McLay 2005; Walker and Holwell 2018). However, body size variation in *I. japonica* males was very small, which was similar to other koinobiont parasitoids because koinobiont parasitoids are less susceptible to host body size than idiobiont parasitoids (Godfray 1994; Harvey et al. 2014). Therefore, differences in body size are not an important factor in *I. japonica* male–male contests.

First arrival males can successfully fight off other males and mate with the female in high percentages. The results suggest that *I. japonica* shows precopulatory mate-guarding behavior. This is to the best of our knowledge the first report of precopulatory mate-guarding behavior performed by a hymenopteran parasitoid. Our observations indicate that the ability of male *I. japonica* to find newly emerging females is an important factor that enhances their reproductive success. Previous studies have demonstrated that the sensitivity of individual males to sex pheromones varied, even within the same populations, and this led to sexual selection by females in lepidopteran insects (Groot et al. 2014). Further studies are needed in order to clarify the main factor that determines the ability of *I. japonica* males to find newly emerging females in the field.

Acknowledgments We are grateful to the members of staff at the Tsukuba Experimental Forest, University of Tsukuba, Japan for their permission to conduct this study on their land. We are also grateful to Dr. Takuya Uehara (National Agriculture and Food Research Organization, Japan) and Mr. Ryousuke Matsushima (University of Tsukuba, Japan) for providing information on the mating behavior of insects. We are grateful to Mr. Teruhito Ishihara (The University of Melbourne) for helping to improve the manuscript.

References

Abe Y (1991) Notices on courtship behaviour and copulation of *Synergus japonicus* Walker (Hym., Cynipidae). J Appl Entomol 111:478–483. https://doi.org/10.1111/j.1439-0418.1991.tb00350.x

Arakaki N, Kishita M, Nagayama A, Fukaya M, Yasui H, Akino T, Wakamura S (2004) Precopulatory mate guarding by the male green chafer, *Anomala albopilosa sakishimana* Nomura (Coleoptera: Scarabaeidae). Appl Entomol Zool 39:455–462. https://doi.org/10.1303/aez.2004.455

Beani L, Dessì-Fulgheri F, Cappa F, Toth A (2014) The trap of sex in social insects: from the female to the male perspective. Neurosci Biobehav Rev 46:519–533. https://doi.org/10.1016/j.neubiorev.2014.09.014

Bennett VJ, Smith WP, Betts MG (2012) Evidence for mate guarding behavior in the Taylor's checkerspot butterfly. J Insect Behav 25: 183–196. https://doi.org/10.1007/s10905-011-9289-1

Brattli MB, Egeland TB, Nordeide JT, Folstad I (2018) Spawning behavior of Arctic charr (*Salvelinus alpinus*): spawning synchrony, vibrational communication, and mate guarding. Ecol Evol 8:8076–8087. https://doi.org/10.1002/ece3.4277

Brockerhoff AM, McLay CL (2005) Comparative analysis of the mating strategies in grapsid crabs with special references to the intertidal crabs *Cyclograpsus lavauxi* and *Helice crassa* (Decapoda: Grapsidae) from New Zealand. J Crustac Biol 25:507–520. https://doi.org/10.1651/C-2548

Chaudhary DD, Mishra G, Omkar G (2017) Strategic mate-guarding behaviour in ladybirds. Ethology 123:376–385. https://doi.org/10.1111/eth.12606

Choi WY, Lee JW, Suh KI (2013) Taxonomic review of the family Ibaliidae (Cynipoidea: Hymenoptera) from Korea. Entomol Res 43:135–141. https://doi.org/10.1111/1748-5967.12015

Christy JH (1983) Female choice in the resource-defense mating system of the sand fiddler crab, Uca pugilator. Behav Ecol Sociobiol 12:169–180. https://doi.org/10.1007/BF00343209

Godfray HCJ (1994) Parasitoids behavioral and evolutionary ecology. Princeton University Press, Princeton

Groot AT, Schöfl G, Inglis O, Donnerhacke S, Classen A, Schmalz A, Santangelo RG, Emerson J, Gould F, Schal C, Heckel DG (2014) Within-population variability in a moth sex pheromone blend: genetic basis and behavioural consequences. Proc R Soc B Biol Sci 281:20133054. https://doi.org/10.1098/rspb.2013.3054

Harvey JA, Harvey IF, Thompson DJ (2014) Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. Ecology 75:1420–1428. https://doi.org/10.2307/1937465

Iltis C, Dechaume-Moncharmont FX, Galipaud M, Moreau J, Bollache L, Louâpre P (2017) The curse of being single: both male and female *Gammarus pulex* benefit energetically from precopulatory mate guarding. Anim Behav 130:67–72. https://doi.org/10.1016/j.anbehav.2017.06.013

Isidoro N, Bin F, Romani R, Pujade-Villar J, Ros-Farré P (1999) Diversity and function of male antennal glands in cynipoidea (Hymenoptera). Zool Scr 28:165–174. https://doi.org/10.1046/j.1463-6409.1999.00013.x

Jarrige A, Kassis A, Schmoll T, Goubault M (2016) Recently mated males of a lek-mating insect intensify precopulatory mate guarding under male competition. Anim Behav 117:21–34. https://doi.org/10.1016/j.anbehav.2016.04.012

Jormalainen V (1998) Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. Q Rev Biol 73:275–304

Kuramitsu K, Kosaki A, Ishihara T, Yamada H, Watanabe K (2016) Infestation of the woodwasp *Tremex apicalis* Matsumura (Hymenoptera, Siricidae) on the large-leaf dogwood *Swida macrophylla* (Wall.) with biological notes on its parasitoid wasps. J Hymenopt Res 52:71–79. https://doi.org/10.3897/jhr.52.10060

Kuramitsu K, Ishihara T, Sugita A, Yooboon T, Lustig B, Matsumori Y, Yamada H, Kinoshita N (2019) The attraction of *Tremex apicalis* (Hymenoptera, Siricidae, Tremecinae) and its parasitoid *Ibalia japonica* (Hymenoptera, Ibaliidae) to the fungus *Cerrena unicolor*. J Hymenopt Res 68:37–48. https://doi.org/10.3897/jhr.68.30372

Longair RW (2004) Tusked males, male dimorphism and nesting behavior in a subsocial afrotropical wasp, *Synagris cornuta*, and weapons and dimorphism in the genus (Hymenoptera: Vespidae: Eumeninae). J Kansas Entomol Soc 77:528–557. https://doi.org/10.2317/E-38.1

Low M (2006) The energetic cost of mate guarding is correlated with territorial intrusions in the New Zealand stitchbird. Behav Ecol 17:270–276. https://doi.org/10.1093/beheco/arj025

Miller AK, Miller PL, Siva-Jothy MT (1984) Pre-copulatory guarding and other aspects of reproductive behaviour in *Sympetrum depressiusculum* (Selys) at rice fields in southern France (Anisoptera: Libellulidae). Odonatologica 13:407–414

Murai M, Backwell PRY (2005) More signalling for earlier mating: conspicuous male claw waving in the fiddler crab, *Uca perplexa*. Anim Behav 70:1093–1097. https://doi.org/10.1016/j.anbehav.2005.02.019

Nordlander G, Liu Z (1994) Review of the family Ibaliidae (Hymenoptera: Cynipoidea) with keys to genera and species of the world. Insect Syst Evol 25:377–392

Quicke DLJ (2014) Phylogeny and systematics of the Ichneumonidae. In: The braconid and Ichneumonid parasitoid wasps: biology, systematics, evolution and ecology. John Wiley & Sons, Chichester, pp 341–449

Romani R, Ishidoro N, Riolo P, Bin F, Fortunato A, Turillazzi S, Beani L (2005) A new role for antennation in paper wasps (Hymenoptera, Vespidae): antennal courtship and sex dimorphic glands in antennomeres. Insect Soc 52:96–102. https://doi.org/10.1007/s00040-004-0780-y

Ruther J (2013) Novel insights into pheromone-mediated communication in parasitic hymenopterans. In: Wajnberg E, Colazza S (eds) Chemical ecology of insect parasitoids. Wiley-Blackwell, Chichester, pp 112–144. https://doi.org/10.1002/9781118409589.ch6

Schubert M, Schradin C, Rödel HG, Pillay N, Ribble DO (2009) Male mate guarding in a socially monogamous mammal, the round-eared sengi: on costs and trade-offs. Behav Ecol Sociobiol 64:257–264. https://doi.org/10.1007/s00265-009-0842-2

Spradbery J (1974) The responses of *Ibalia* species (Hymenoptera: Ibaliidae) to the fungal symbionts of siricid woodwasp host. J Entomol Ser A, Gen Entomol 48:217–222. https://doi.org/10.1111/j.1365-3032.1974.tb00058.x

van den Assem J, Gijswijt MJ, Nübel BK (1980) Observations on courtship - and mating strategies in a few species of parasitic wasps (Chalcidoidea). Netherlands J Zool 30:208–227. https://doi.org/10.1163/002829679X00386

Wada S, Ashidate M, Goshima S (1997) Observations on the reproductive behavior of the spiny king crab *Paralithodes brevipes* (Anomura: Lithodidae). Crustac Res 26:56–61. https://doi.org/10.18353/crustacea.26.0_56

Wada S, Tanaka K, Goshima S (1999) Precopulatory mate guarding in the hermit crab *Pagurus middendorffii* (Brandt) (Decapoda: Paguridae): effects of population parameters on male guarding duration. J Exp Mar Biol Ecol 239:289–298. https://doi.org/10.1016/S0022-0981(99)00045-3

Walker LA, Holwell GI (2018) The role of exaggerated male chelicerae in male–male contests in New Zealand sheet-web spiders. Anim Behav 139:29–36. https://doi.org/10.1016/j.anbehav.2018.02.020

Wilson F (1961) Adult reproductive behaviour in *Asolcus basalis* (Hymenoptera: Scelionidae). Aust J Zool 9:739–751