Chapter 3
Mating Behavior

Lisa Filippi, Mantaro Hironaka, and Shintaro Nomakuchi

Abstract The mating behaviors of *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) were observed under both natural field conditions and manipulated field conditions. The mating system is best described as scramble competition polygyny. Males terminate reproductive diapause before females. They fly about the aggregations, land on one, and immediately evert the aedeagus and attempt copulation. Females do not fly and reject all attempts by males to mate. Males and females have both short- (<3 min) and long-term (up to 1 h) copulations, and long-term copulations are much more effective at inseminating females. They mate both end to end (short term) and in a V-pattern (long term). Both males and females mate multiply, but females typically have only one long-term mating. Females that had long-term copulations had slightly more advanced ovarian development and were more likely to be roaming near but not in the aggregations. Males achieving long-term matings were not larger than unsuccessful males. We discuss the possibility of cryptic female choice.

Keywords Cryptic female choice · Fat body · Female resistance · Intrasexual competition · Mating strategies · Mating success · Ovarian development · Promiscuity

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3.1 Introduction

One of the most bizarre observations we made soon after initiating this research was the comical antics that occurred during the mating season. Male *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) would fly erratically about the aggregation site, dropping down to, and rapidly scurrying about on, different sedate clusters, seemingly trying to wake everyone up. And the landing was not at all skillful; the males just appeared to fall out of the sky and happen to artlessly land on a cluster, which was easy to do when the foliage on the trees was covered with the clustered bugs. Once a male had landed, he everted his aedeagus and backed it into the abdomen of any bug he encountered, whether male or female. Once joined end to end, his legs thrusted rhythmically, a movement that appeared to be consistent with ejaculation. The entire time the male was attempting to copulate, and even well after he had succeeded in inserting his aedeagus, his partner, male or female, continued to rock its body from side to side, kicking him with its hindlegs and running away, even after they were attached. Of course, the goal of the male should be to meet with some measure of reproductive success if repeated often enough, and this behavior was repeated numerous times over the remaining 2 weeks of the male's life. Males were clearly polygynous, attempting to maximize their reproductive success by mating with as many females as possible. Nonetheless, we recognized early on that despite living in predominantly sedate aggregations where half of his cluster mates are female should preclude the need to search for a mate, the dogged reluctance of females means that securing a mate is still a costly endeavor for a *P. japonensis* male. So, what was going on here? And why were females so invariably reluctant to mate when the goal of their existence should also be to reproduce? We undertook a series of studies between 1990 and 1995 and reported the results in two papers. The goal of the first paper was to characterize the behavioral and ecological parameters and the physiological conditions that prevail under natural conditions during the mating season of *P. japonensis* (Tsukamoto et al. 1994). The second series of studies also took place in the field, but involved individually marked and measured bugs, and containment such that we could observe every mating a female had (Filippi et al. 2000). In an effort to share our journey of discovery with the reader, we will introduce the findings of the two studies in sequence. As polygyny was the most conspicuous aspect of the mating system we observed, we begin by briefly considering the factors that promote the different types of polygyny.

3.2 Mating Systems That Involve Polygyny

Polygyny is common in insects and other terrestrial arthropods, and their mating systems typically fall into one of three categories: female defense polygyny, resource defense polygyny, or scramble competition polygyny, and polygyny of all types is particularly common in the Heteroptera (Thornhill and Alcock 1983). The specific mating system is determined by the environment that the insects inhabit; the dispersion and abundance of resources, which could be food, nests, territories, oviposition sites, etc.; and the insects' abundance and dispersion in the habitat (Emlen and Oring 1977; Alcock 1980; Dickinson 1992; Choe and Crespi 1997). Because all of these conditions can vary over the course of a season, temporal variation in social and environmental conditions can cause a shift in the mode of polygyny (McLain 1992). For example, males of the neotropical harvestman *Acutisoma proximum* (Arachnida: Opiliones) initially aggressively defend territories from other males (resource defense polygyny). The territories contain particular vegetation that females are attracted to for oviposition sites. However, later in the reproductive season, males stop patrolling and defending territories and sequentially defend individual females that will oviposit in their territory (Buzatto and Machado 2008).

3.2.1 Female Defense Polygyny

Female defense polygyny is predicted when a population is male-biased and/or there is a likelihood of sperm displacement (Parker 1970), as in the aggregating tropical bug, *Dysdercus bimaculatus* Stål (Pyrrhocoridae; Carroll and Loye 1990); the soapberry bug, *Jadera haematoloma* Herrich-Schaeffer (Rhopalidae: Carroll 1991); and the ambush bug, *Phymata fasciata* Gray (Phymatidae; Dodson and Marshall 1984). Post-copulatory guarding of females that mate multiply is an effective means to mitigate sperm competition because sperm precedence patterns in insects indicate that the last male to mate with the female is favored to fertilize a significantly greater proportion of the eggs (Walker 1980). Female defense polygyny is costly to males because they forfeit other mating opportunities while guarding a female. However, in high-density, male-biased aggregations where a female could remate quickly and where sperm displacement is likely to occur, guarding would be more likely to increase the male's reproductive success than searching for another female (Carroll and Loye 1990; Carroll 1991; Schöfl and Taborsky 2002). Males can gain an advantage from guarding a female even when the guarding does not continue until oviposition, as in *Megacopta punctissima* Montandon (Plataspidae; Hibino 1985; Hosokawa 2009), where there is a 24-h cycle for mating, and females have a 7-day oviposition pattern. Most matings occur during the 20 h between 2:00 pm and 10:00 am the following day. Average copulations lasted 10 h, and males remaining with the female for longer than 4 h were considered to be engaging in mate-guarding because sperm transfer occurs between 2 and 4 h of copulation. Thus, any guarding beyond what is needed for sperm transfer should enhance sperm competition.

3.2.2 *Resource Defense Polygyny*

Resource defense polygyny is predicted when females are attracted to a resource, which could be food, a foraging or nesting territory, or an oviposition site that a male can defend (Emlen and Oring 1977). Limited resources can be defended by high-quality males, and females are attracted to the defended territory, leading to a reproductive skew. The strength of sexual selection and the intensity of contests are increased with reproductive skew, and this leads to the evolution of exaggerated traits (Emlen 2014; Herberstein et al. 2017), such as the large mandibles used by males of the tropical rove beetle, *Leistotrophus versicolor* (Coleoptera: Staphylinidae) (Forsyth and Alcock 1990). Male size varies widely and so does the size of their mandibles, which they use to aggressively displace other males from dung pats. Dung pats are foraging sites that females are attracted to, although the resource is actually the dipterans that are attracted to the dung. In the Heteroptera, nonterritorial polygyny is the norm and occurs in many species, including *Euschistus conspersus* Uhler, Pentatomidae (Alcock 1971), and the infamous *Nezara viridula* Linnaeus, Pentatomidae (McLain 1981), but there are four exceptions. *Acanthocoris sordidus* Thunberg, (Fujisaki 1980, 1981); *Acanthocephala femorata* Fabricius (Mitchell 1980); *Neacoryphus bicrucis* Say (McLain 1984, 1986, 1992); and *Notobitus meleagris* Fabricius (Coreidae; Miyatake 1995) all display resource competition polygyny. In these species, the resource occurs in patches that are defendable by the male.

3.2.3 *Scramble Competition Polygyny*

On the other hand, a variety of ecological parameters promote the evolution of scramble competition polygyny. Patterns that range from the situation where many potential mates or resources are indefensible because they are widely scattered over time and space to that where there is an abundance of potential mates gathered in a small and accessible area, as is the case with *Parastrachia japonensis*, promote the evolution of scramble competition polygyny (Thornhill and Alcock 1983; Nishida 1989, 1993; Herberstein et al. 2017). Inherent in the premise of scramble competition polygyny is the concept that little or no direct male-to-male aggression or female defense is involved (Thornhill and Alcock 1983; Herberstein et al. 2017). This type of polygyny aligns best with what we observe in *P. japonensis*.

3.3 Mating System in *Parastrachia japonensis*

The bugs emerge from their underground winter hibernation in late February, early March, and relocate to the aggregation site. As described in previous chapters, the aggregation site comprises small perennial trees and plants, primarily *Eurya* *japonica*, and ferns within ten meters of the host trees, and it is at this site, in late April, that the mating behavior begins. We first observed the overall mating behavior at the aggregation site during the reproductive season of 1992 in an attempt to characterize the mating system and gain an understanding as to how males and females are optimizing their reproductive success (Tsukamoto et al. 1994).

3.3.1 Flight by Males and Mating Behaviors in the Field

Just prior to the start of the mating season in late April of 1992, the trees, shrubs, and plants at the aggregation site were laden with the flamboyant red and black clusters of bugs that were still fairly sedate. The focal subpopulation for our observations in 1992 consisted of about 1300 individuals in clusters on a Japanese viburnum bush (*Viburnum japonicum*; ~2m^2). We positioned ourselves such that we could observe the entire aggregation site unobtrusively and logged 34.5 h of mating behavior over the 19-day mating season from April 25th to May 14th, with an average of 2.0 h of observations per day (range 0.5–4.0 h). The temperature during the mating season ranged from 15.5 to 26.5 °C. The initial sex ratio of the clustered individuals was 1:1. By the third week in April, males began to leave the clusters and walk about the aggregations, occasionally stopping to engage in a behavior we have termed "genital grooming" (Fig. 3.6a). They everted the aedeagus and rubbed it with the hindlegs for a few minutes before they moved on. Males did not become active simultaneously; rather, they seemed to gradually awaken from their reproductive diapause, with increasing numbers of males moving about the aggregation site daily, first walking and then flying in a somewhat circular pattern about the aggregation site. We observed over 7600 flights, and all were brief, lasting under 10 s. It is significant that only males flew, probably as a means to gain access to as many females as possible, while females only walked, conserving their energy for the costly parental care behaviors. Males flew from just a few centimeters to 10 m or more but rarely strayed more than 2 m from the aggregations until most of the females had relocated to the host tree. They were certainly not graceful in flight and were even clumsier on landing, appearing to tumble haphazardly out of the sky and fall onto the clusters. The clusters were so densely arranged at the aggregation site that the neurological investment in more skillful flight and landing behaviors was apparently deemed unnecessary by evolutionary forces. The number of flights observed per hour increased dramatically as the season progressed and then dropped off precipitously in early May before increasing again and peaking at 750 flights per hour near the end of the mating season (Fig. 3.1a; Tsukamoto et al. 1994). The dip in the second week of May was likely due to a spell of low temperatures as the frequency of flight behavior during the mating season was clearly temperature dependent (Fig. 3.1b, Tsukamoto et al. 1994). Sixty seven percent of all flights occurred in the temperature range of 22–24 °C, although only 47% of the observations took place in that range.

Further, in 1992, no flights were observed below 19 °C. Light rain, indicated by asterisks in Fig. 3.1a, apparently had no effect on flight behavior. Males alighted on

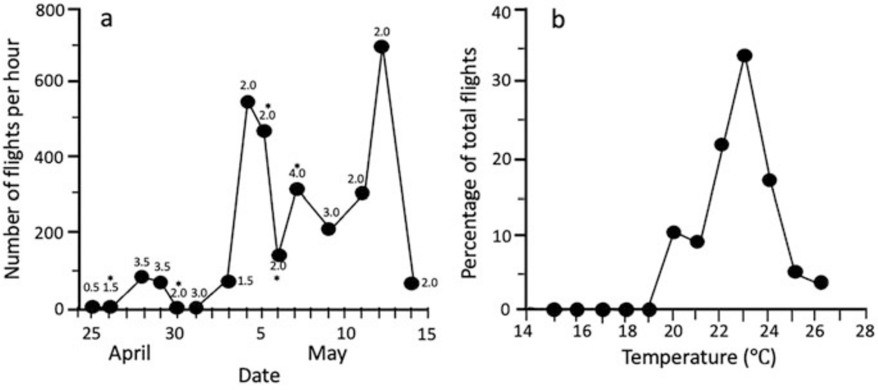

Fig. 3.1 Flight frequency of males (**a**) throughout the mating season and (**b**) as a function of temperature. Numbers above dots represent hours of observation; asterisks indicate rainy days. Adapted from Tsukamoto et al. (1994)

the clusters in an apparent attempt to "wake up" the females. There was no courtship behavior at all. In fact, when a male landed on a cluster of bugs, he rapidly everted his aedeagus and, backing up, immediately attempted to insert it into the abdomen of whatever individual happened to be closest to him, whether male or female. Interestingly, both males and females resisted the male's attempt at intromission, though males resisted more intensely. They kicked out at him with their hindlegs, rocked their bodies, and attempted to run away, as the male feverishly continued to attempt intromission. If successful, the copulation was most often end to end, as is common in stink bugs, but also occurred as a V-pattern, with the male grasping the female across the dorsum with his front and midlegs (Fig. 3.6b). In both postures, the male displayed a rhythmic jerking movement with his legs and abdomen soon after intromission was achieved which suggested ejaculation was taking place. We categorized this mating system as scramble competition polygyny, though, as will be described later, there was also behavior that indicated intrasexual competition by males.

3.3.2 Copulation Frequency and Success Rate by Males in Aggregations and at Isolated Sites

From May 4 to 11, we observed an average of 59 copulations per hour despite that during this period the overall number of bugs at the site had dropped to nearly one fourth the original number (see Sect. 3.6). This suggests that the number of matings per male actually increased over time. Copulation occurred with individuals within aggregations and with those that had walked away from the aggregations and were isolated on nearby branches. Suspecting that bugs remaining in the aggregations were not yet receptive to mating, we compared the rate of successful intromission (copulation) by males attempting to mate within an aggregation and at an isolated site near the aggregations. Among 1281 copulations observed, 45% occurred in aggregations and 55% occurred in isolation. Near the end of the mating season, up to three times as many matings occurred with isolated females (Fig. 3.2a; Tsukamoto et al. 1994). Males had a significantly greater chance (12%) of successfully mating with a female when the attempt was with an isolated female (Fig. 3.2b; Mann-Whitney U test, $U = 81$, $p < 0.001$). Despite the intense rejection males encountered, greater than 70% of their attempts to mate were ultimately successful.

3.3.3 Duration of Copulation in Aggregated and Isolated Conditions

As previously mentioned, copulations were both end to end and in a V-shaped pattern with the male on top, grasping the female across the dorsum. Both males and females mated repeatedly, but females always resisted initially. Of the 1281 copulations observed in 1992, 987 were timed. We noted two types of copulation based on duration, short term (ST) 2–100 s and long term (LT) 3–57 min, and the duration was correlated with mating posture. ST matings tended to be end to end, and LT matings were in the V-shaped pattern. In 1992, we did not observe copulation durations between these two ranges. All copulations that took place within the aggregations and at isolated sites near the aggregations during the observation period on a given day were recorded. 93% of all timed copulations were ST and averaged 14.9 ± 10.3 s (mean ± SEM, $n = 922$; Fig. 3.3a, Table 3.1; Tsukamoto et al. 1994). As with flight, the duration of copulation was temperature dependent (Fig. 3.3b); there was a significant negative correlation between temperature and copulation duration (Pearson correlation coefficient, $r = 0.953$, $p < 0.001$). It is likely that insemination takes longer at lower temperatures because of the greater metabolic requirements of muscles required for ejaculation and insemination. Long-term copulations averaged 22.6 ± 14.7 min (mean ± SEM, $n = 64$; Fig. 3.3c; Table 3.1), and the incidence of LT matings increased markedly after May 5th (Fig. 3.3c) when female numbers at the aggregation/mating site dropped by nearly 50% (Fig. 3.4). This suggested that the LT matings might represent a switch from simple scramble competition polygyny to guarding behavior, as described for *N. viridula* (McLain 1981), once intromission was achieved in response to the reduced operational sex ratio (OSR). While there was no difference between the numbers of ST copulations that occurred within an aggregation and those at isolated sites, there were significantly more LT copulations at isolated sites (Table 3.1; Fisher's exact test, $p < 0.00001$). There was no significant difference between the duration of ST or LT matings that occurred within an aggregation or in isolation, although LT matings occurring in isolation tended to be longer.

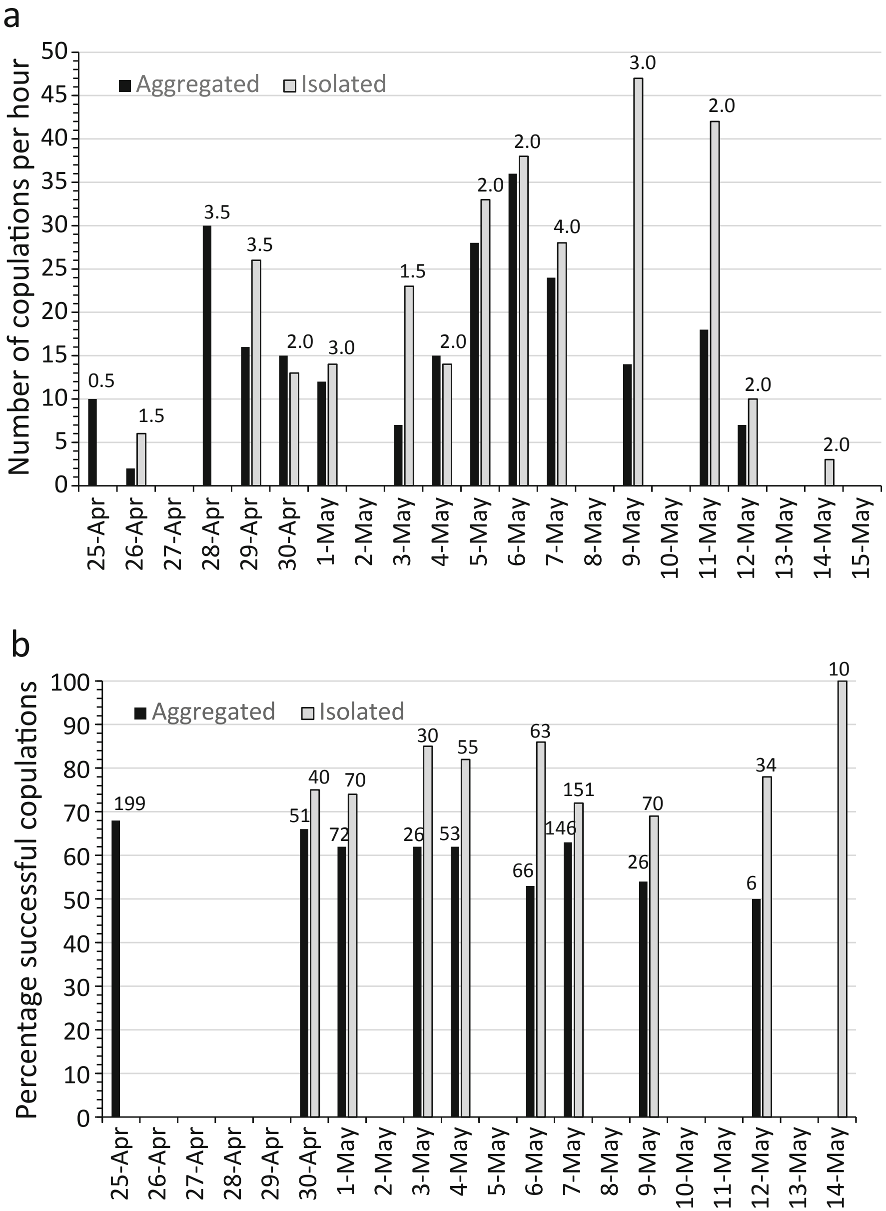

Fig. 3.2 Copulation success and frequency in aggregated and isolated *P. japonensis* throughout the mating season. (**a**) Number of matings observed in aggregations and in isolated conditions per hour. Numbers above columns represent number of hours of observation on that date. (**b**) Percentage of mating attempts by males that were successful in aggregations and isolated conditions on each date. Numbers above bars represent number of mating attempts under each condition. Adapted from Tsukamoto et al. (1994)

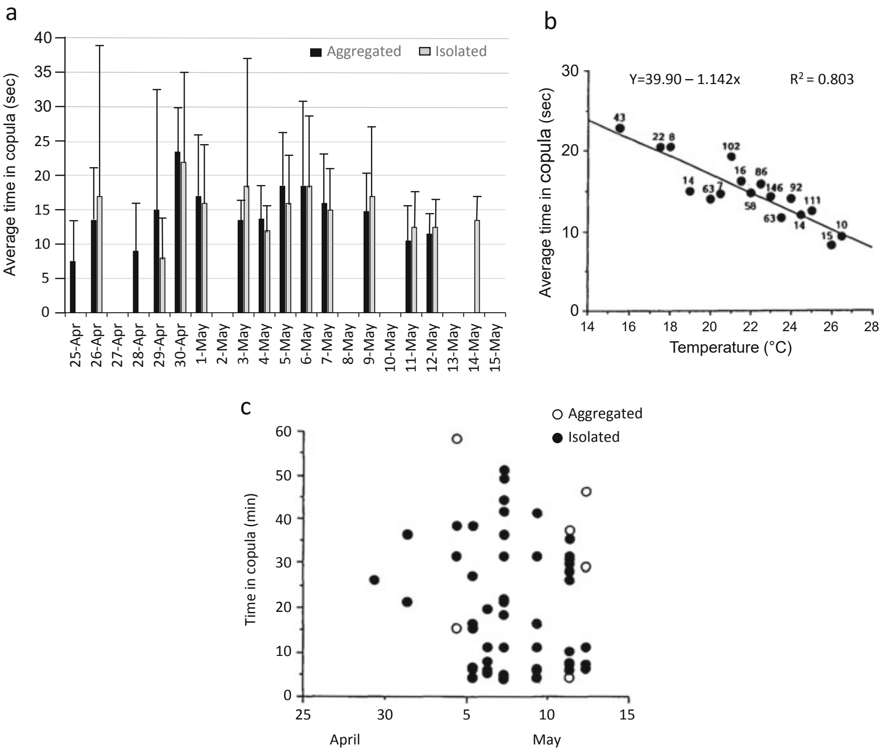

Fig. 3.3 Duration of copulations in *P. japonensis.* (**a**) Duration of short-term copulations (2–100 s) throughout the 19-day mating season. Error bars represent S.E.M. (**b**) Impact of temperature on copulation duration. $P < 0.001$. (**c**) Duration of long-term matings throughout the mating season (3–57 min). Comparison of number of matings in aggregation vs isolated for short-term and long-term matings, Fisher's exact test, $p < 0.00001$. Adapted from Tsukamoto et al. (1994)

Table 3.1 Duration of short- and long-term copulations of *P. japonensis* in aggregations and in isolated conditions in 1992. Adapted from Tsukamoto et al. (1994)

Mating	Parameter	Aggregated	Isolated	Overall	
Short term	*n*	455	467	922	
	Mean duration, s ± SEM	13.6 ± 5.2	13.6 ± 3.6	14.9 ± 10.3	
	Range, s	2–100	2–80	2–100	
Long term	*n*	6	58	64	Fisher Exact test $P < 0.000001$
	Mean duration, min ± SEM	30.5 ± 19.9	21.9 ± 14.0	22.6 ± 14.7	
	Range, min	3–57	3–50	3–57	

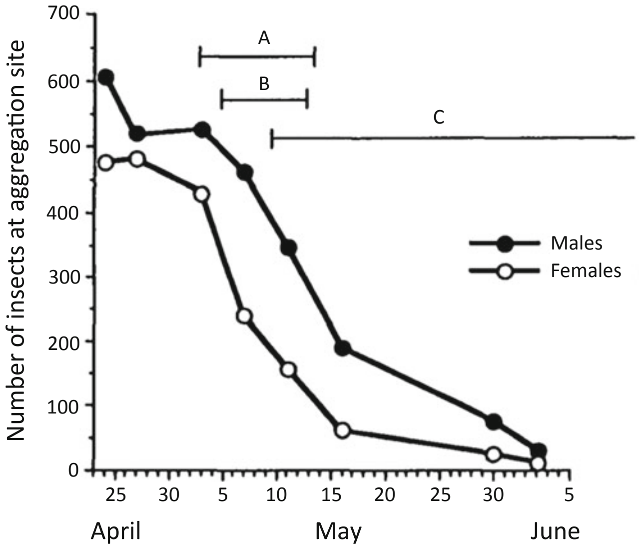

Fig. 3.4 Change in numbers of males and females at the aggregation site during the mating season. Horizontal bars indicate (**a**) period of peak number of male flights; (**b**) peak number of copulations; (c) period that females were relocating to host tree

3.3.4 Change in Numbers of Males and Females at the Aggregation Site over the Mating Season

Although most of the observations for the 1994 paper were from the mating season of 1992, we collected data on the pattern of dispersal from the aggregation site at the same site in 1993. In 1993, the size of the subpopulation at that site was similar to 1992; however, the initial sex ratio differed (57% male, 43% female). As males continued to fly about the aggregation site mating with females, some females seemed to lose their response to the aggregation pheromone and began to relocate to the host tree as lone individuals. At this point (early May), the drupes on the host tree were still very immature, so females did not begin feeding but walked along the branches of the host tree. As the season progressed, the sex ratio at the aggregation site became markedly male biased (Fig. 3.4), although the numbers of both sexes decreased dramatically as more and more females moved on to the host tree and males died from depletion of their fat body reserves (unpublished data). By May 7th, the sex ratio (M:F) was 66:34, and by May 11th, there were half as many bugs as the initial number, and the sex ratio was 70:30. By May 14th, nearly 76% of the remaining individuals at the aggregation site were males. By June 2nd, only 40 individuals remained, 30 of which were males. These 40 individuals did not mate that year and remained in the aggregation until they were joined by the new adults that emerged in late July/early August. Females that had relocated to the host tree were first noted feeding about 10 days after they began moving to the host tree; they fed on the bits of endosperm in the as yet unripe and green drupes. Males, including the few that had followed the females, never fed on the host tree and, thus, never fed after becoming adults the previous summer or two summers earlier.

3.4 Ovarian Development and Fat Body Content Throughout the Mating Season

Because *P. japonensis* enter reproductive diapause after emerging as adults, we were curious to understand at what point their reproductive organs become sexually mature and also how their fat body stores are used. In 1990, we collected females from different sites, aggregated and isolated, at different points during and soon after the mating season and dissected them to assess their reproductive development. We also examined fat body content in individuals at different stages of ovarian development. As the dates of the mating season are fairly consistent from year to year, we attempted to correlate that data with the behavioral data we collected in 1992. The data indicate that ovarian development was staggered among the females and did not progress beyond the earliest stage of previtellogenesis unless the female was inseminated (Fig. 3.5, Tsukamoto et al. 1994). This suggests that insemination might be triggering the initiation of ovarian development. One isolated female on June 7 still had undeveloped ovarioles, although she was inseminated. Since this date was well after all males had already died, that particular female's undeveloped yet inseminated state was a curious anomaly. Remarkably, the data for fat body content revealed that even after 9 months, and in many cases 1 year and 9 months, 74% of females still maintained the maximum level of fat body content. Fat body content decreased with progression of ovarian development. While 12% of females with early previtellogenesis had depleted fat body content, the majority had a fair amount of fat bodies remaining, nearly 90% of females in ovarian stage 2 (late previtellogenesis) had depleted their fat bodies to Factor 1 or 2, but fully 41% of those in the latest stage of ovarian development (vitellogenesis and beyond) had a fat body Factor of 3. This suggests that females utilized their abundant fat body reserves stored during the nymphal stage in the previous spring (or two springs prior!) for the earlier stages of ovarian development over ~10 days that they remained on the host tree without feeding and then fed as their stores dwindled in order to achieve full egg production. After about 10 days of actually feeding on the host tree, females descended the tree and oviposited under the leaf litter typically several meters beyond the crown of the host tree (Tsukamoto and Tojo 1992; Filippi-Tsukamoto et al. 1995).

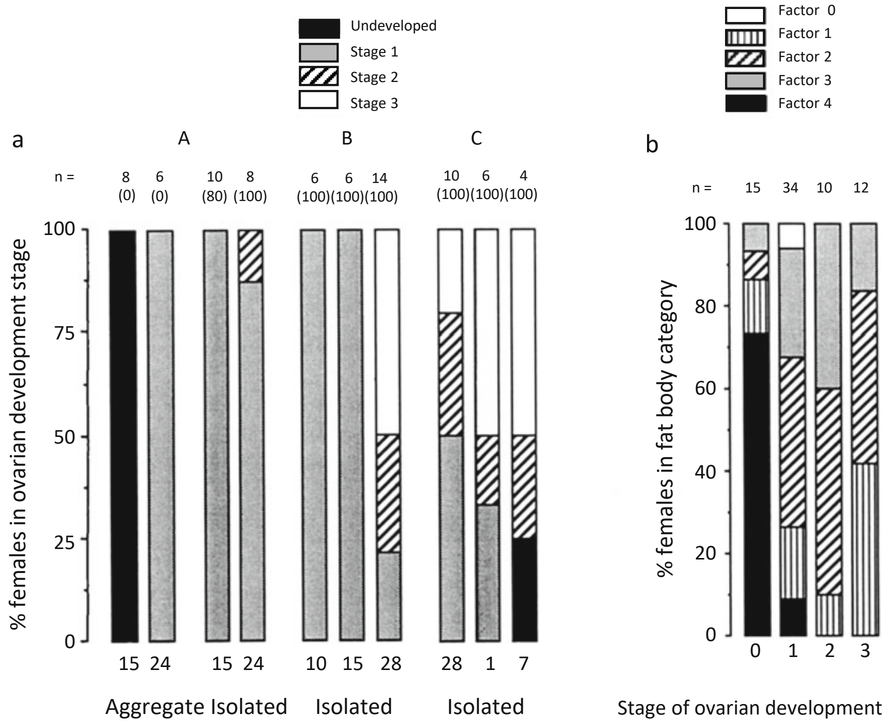

Fig. 3.5 Ovarian development and nutritional status of *P. japonensis* females during the mating season. (**a**) Ovarian development at three different sites during the mating season as determined by the state of the largest of eight unilateral ovarioles. Stage 0, undeveloped; stage 1, early/mid-previtellogenesis; stage 2, late previtellogenesis; stage 3, vitellogenesis to mature eggs. Numbers in parentheses indicate percentage of inseminated females. Aggregated and isolated females indicated. (**b**) Fat body content and stage of ovarian development. Subjects were lone females collected from host tree from May 15 to June 7 (mating season ended May 15). Adapted from Tsukamoto et al. (1994)

3.5 Male Intrasexual Competition

While the predominant mating system is clearly scramble competition polygyny, with males competing indirectly to gain access to as many females as possible, several other behaviors suggestive of more direct male intrasexual competition were observed. We considered the possible advantage of the V-posture as opposed to the end-to-end posture. This posture was more common in the LT matings, and we considered whether it might be a form of mate guarding (Parker 1970). However, because females do not oviposit until about 2 weeks after mating and have ample time to mate with other males in the interim, we concluded that in the absence of evidence that insemination after the mating was impossible, guarding as a function of the LT mating was unlikely.

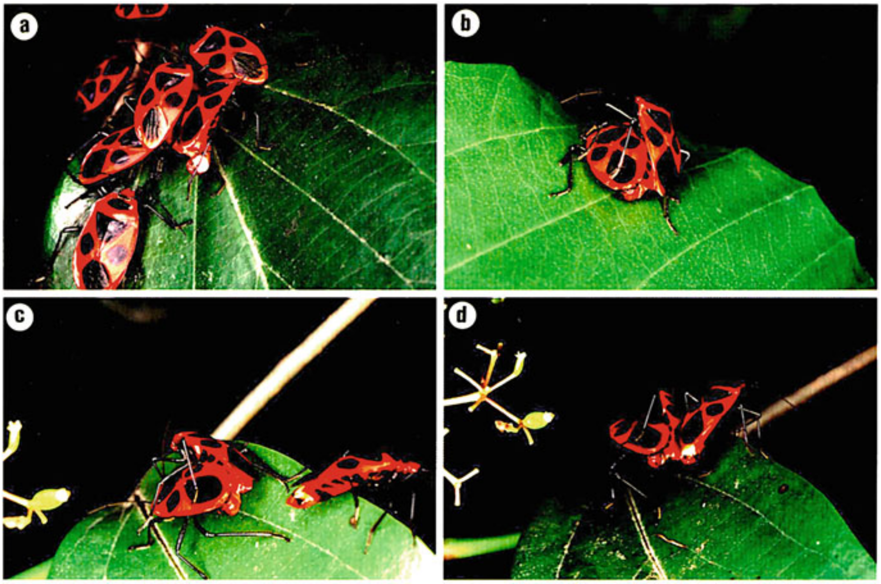

Fig. 3.6 Copulation behavior in *Parastrachia japonensis*. (**a**) Male everting the aedeagus and engaging in "genital grooming"; (**b**) V-pattern copulation with male on top, grasping female across the dorsum; (**c**) male intruding on a mating couple; (**d**) successful displacement of original male and intromission by intruding male. Adapted from Tsukamoto et al. (1994)

Another male behavior that indicated direct competition was that of males intruding on V-pattern mating couples in an attempt to dislodge the engaged male. Intrusions were observed on 121 occasions (9.4% of all mating attempts; Fig. 3.6c). The intruder would back into the joined abdomens, kicking at the male in an attempt to dislodge him and simultaneously attempting intromission with the female. 16 (13.2%) of these intrusions resulted in the original male being displaced and the intruder successfully mating with the female (Fig. 3.6d). Males were also seen attempting to mate with other males on 84 occasions and met with success 12 times (14.3%). The couplings were invariably end to end, complete with the thrusting phase suggestive of ejaculation (Tsukamoto et al. 1994). As no studies on sperm precedence have been done on this species, the impact of these behaviors on male reproductive success remains unclear, but they certainly warrant further scrutiny. In particular, information on how the change in OSR at the aggregation site impacts these male behaviors would contribute to our understanding of their significance in male reproductive success. OSR changes considerably as the mating season progresses because, as described above, females eventually relocate to the host tree after mating; however, most males remain at the aggregation site trying to mate with the remaining females. This skew in OSR suggests that competition between males likely becomes intense (Emlen and Oring 1977; Shuster 2009). Moreover, females already engaged in an LT copulation (V-pattern) might be more receptive to mating and emitting more pheromones and thus be more attractive to males who then try to dislodge and replace an already engaged male.

With regard to same sex mating between males, which is quite common in insects (Monk et al. 2019), Lerch and Servedio (2021) present a model that supports the hypothesis that same-sex mating can evolve if conditions favor indiscriminate mating as the optimal strategy. Further, Monk et al. (2019) present a convincing argument that indiscriminate mating might not be the conundrum it appears to be and is in fact the ancestral state. The authors suggest that it had not been selected out because the cost of possible missed different sex copulations is greater than the cost of indiscriminately engaging in same-sex mating. The densely aggregated arrangement of *P. japonensis* at the aggregation/mating site, and the likelihood that pheromones from one sex end up on the other, confusing the males further, suggests that the same-sex mating that occurs between males in this species likely represents a classic case of mistaken identity in the optimal strategy of indiscriminate mating (Scharf and Martin 2013; Monk et al. 2019).

3.6 Conclusions of Initial Mating Study

Through solely observing behavior in the field, without individual identification, we found that males become active first, in late April, early May; they wander around the aggregations, occasionally stopping to engage in “genital grooming.” Only males engage in flight, and this seems to facilitate males gaining access to as many females as possible. Males mate with females in clusters at the aggregation site and with isolated individuals near the aggregations, as well. The mating system can best be described as scramble competition polygyny (Thornhill and Alcock 1983). Females resist all attempts by males to mate, even after intromission has occurred, and copulations are in both the end-to-end posture, and in the V-posture, with the male on top, grasping the female across her dorsum. Both postures are accompanied by a rhythmic thrusting motion by the male as soon as intromission is achieved. Mating with regard to the sex of the partner appears to be indiscriminate, probably because for males the strategy of occasionally mating with a male during the chaotic scramble is less costly than the missed mating opportunities that might occur otherwise. Both males and females mated repeatedly. Both short-term and long-term matings were observed, the long term being more associated with the V-posture. Short-term matings are much more prevalent, and long-term matings are more common later in the mating season. As females mate, they gradually move over to the host tree, and the sex ratio at the aggregation site becomes decidedly male biased, likely intensifying competition between males. Ovarian development does not begin in earnest until after insemination has taken place, and the earliest stages of ovarian development are achieved with the fat body reserves from the nymphal stage, before the females actually begin feeding.

3.7 Observations of Manipulated Mating Behavior in the Field

Our goal of the next series of studies was to sort out the significance of the different mating durations, ST and LT. The “thrusting” behavior by males as soon as intromission had been achieved appeared to be consistent with ejaculation, so we assumed sperm was being transferred in both ST and LT matings. However, in 1993, LF did a preliminary investigation into this, dissecting several females that had just had either an ST or an LT copulation in the field. Of course, it was unclear whether the females had mated previously; however, among the females examined, sperm were only found in females that had had a long-term copulation. This was curious and led us to undertake a semi-natural study in the field. In both 1994 and 1995, 1 month before males and females became active, females were individually marked with liquid paint markers, their weight was recorded with a digital balance, and their pronotum width was measured with a digital calipers. The virgin females were enclosed in mesh bags (50 cm × 80 cm) on branches of a *Eurya japonica* tree, which prevented any unobserved mating behavior. We attempted to also enclose males, but because they expended so much energy trying very hard to fly in the bags, they did not survive to mate, so thereafter we collected males on the date that they were used for the mating trials. Mating trials were carried out in the field in three-tiered mesh cages suspended on a pole between two trees. Each of the three compartments was 50 cm × 30 cm × 25 cm (L × W × H). To avoid physical contact across the mesh between the bugs in adjacent compartments, either only the top and bottom compartments were used, or contact was prevented by separating adjacent chambers with cardboard dividers. Males were extremely fragile when it came to being manipulated in any way once they had initiated mating behaviors for the season. Recall that they do not feed, and with all the flight activity, they burned through their fat reserves fairly quickly and seemed to die off faster in the mesh cages than they did in the field. Our objective was to determine whether there was a discrepancy in insemination rate between ST and LT matings, so we just kept adjusting the sex ratio (M:F) to ensure that there were enough males that were vigorous enough to mate with the females in the cages. Thus, the sex ratios ranged from 1:2 to 2:1 in 1994 and, to increase the sample size of mated females, from 2:1 to 3:1 in 1995. Though there might have been some impact of sex ratio, for the purposes of this study, we assumed that there was no impact of sex ratio on insemination success of ST and LT matings and pooled data for all sex ratios.

In 1994 we carried out observations for a total of 23.3 h on 8 days throughout the mating period from May 8 to May 27, averaging ~174 ± 36 min per day, and in 1995 observations totaled 34.6 h on 14 days from May 5 to May 30, averaging 148.2 ± 36 min per day. Males and females were introduced to the chambers at the start of each observation session, and, after one or several copulations, females only were either returned to their bags and set back on the *Eurya* branches; transferred to the laboratory for dissection to determine stage of ovarian development, fat body abundance, and insemination rate; or enclosed in mesh bags on the host tree so they could feed. Those females were then dissected after feeding for 11–58 days. We documented the sequence and duration of each female's copulations and recorded their post-copulatory weight.

3.8 Patterns of Short- and Long-Term Matings over the Mating Season

In 1994, 160 copulations were observed for 95 females. In 1994 we did not record the number of females that did not mate. In 1995, among 138 females, 85 females engaged in 154 copulations, and 53 females did not mate at all. Although male's flight was constrained in the mesh cages, and we suspect that this "frustration" contributed to their early demise, the actual copulations proceeded as described for the bugs in the natural aggregation in the field. Males everted the aedeagus, backed into the female's abdomen, and attempted intromission. Females rejected males by rocking the body, kicking them with their hindlegs and running away, even after intromission had been achieved. The rhythmic thrusting behavior by males began 4–5 s after intromission and continued until about 10 s, while in the end-to-end position (stage 1). The male then tried to grasp the female across the dorsum with his front and midlegs, rotating his head dorsolaterally. Only if this maneuver was successful did the mating proceed to the V-pattern posture (stage 2) and become a long-term copulation.

Even while in the V-pattern posture the linked couple would intermittently walk about the cage, always with the female dragging the male along and occasionally rocking her body in an attempt to dislodge the male. This behavior was more prevalent after about 15 min in copula. ST matings were terminated by the female, but LT matings appeared to be terminated by the male. He disengaged the aedeagus, engaged in genital grooming for a few seconds, and then went off to seek another mating. Stage 1 and stage 2 postures are illustrated in Fig. 3.8.

In 1995, individual females were given multiple opportunities to mate, but only on one day, during one observation session. The seasonal changes in the frequency of ST and LT matings in 1995 demonstrate that the frequency of ST matings decreased over the season and that of LT matings increased; there were females who did not mate at all at every stage in the mating season. Short-term matings comprised 75% of all matings in the early phase of the mating season (Fig. 3.7a). Less than 10% of the females had LT matings, and about 23% of the females did not mate early in the season. By the middle of the mating season, the percentage of females experiencing ST matings dropped by about 30% to about 45%, and the percentage of females that had LT matings increased to about 35%, while the percentage of females who did not mate at all increased by 17% to about 40%. Late in the season only about 25% of females had ST matings, fully 50% of females experienced an LT mating, and the same proportion (40%) did not mate at all. The data suggest that as the mating season progressed, females were more willing to accept the LT mating. The data for 1994 followed a similar seasonal pattern.

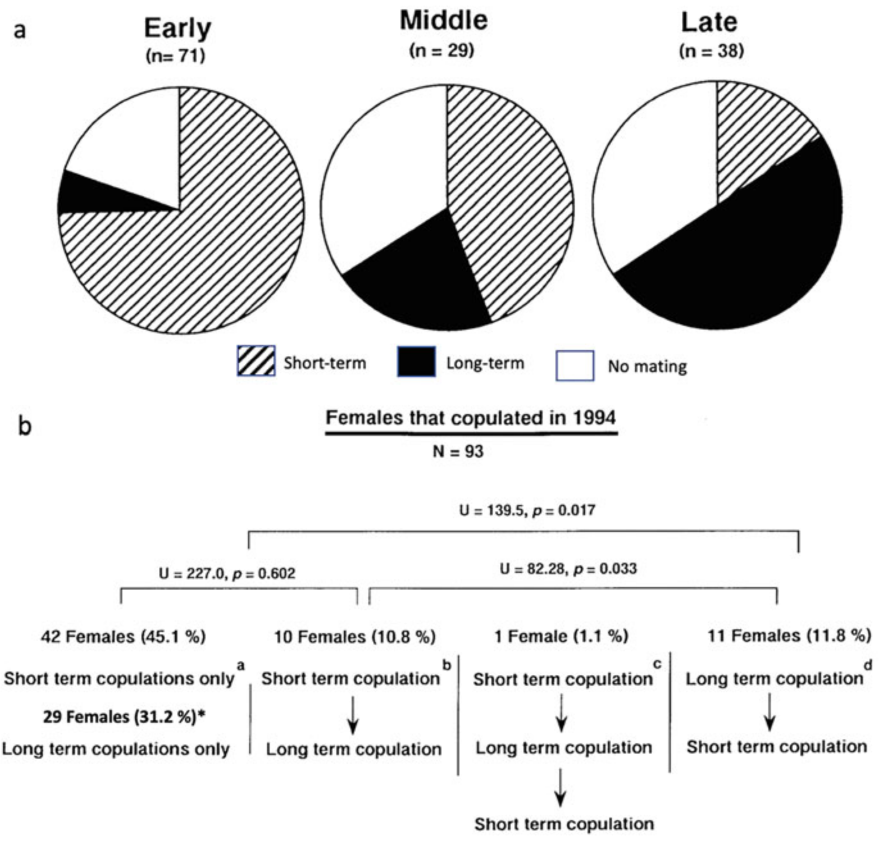

Fig. 3.7 Frequency and pattern of short- and long-term matings during the mating season. (**a**) Seasonal changes in numbers of females that experienced short-term, long-term, or no matings over the course of the mating season in 1995. Females were allowed multiple opportunities to mate, but only on one day of observation. (**b**) Pattern of short- and long-term matings in females who were given multiple opportunities to mate on multiple days during the mating season of 1994. Females that did not copulate are not included in the figure. (*) One of these females had two long-term copulations. (a) 1–7 short-term copulations (ST) only; mean 1.5 ± 1.7 (S.D.); (b) 1–2 ST before long-term copulation (LT); mean 1.2 ± 0.42; (c) 2 ST before LT, 1 ST after LT; (d) 1–7 ST after LT; mean 2.5 ± 2.1. Mann-Whitney U test for significance of number of ST among the three groups that experienced ST. Adapted from Filippi et al. (2000)

3.9 Frequency, Duration, and Insemination Success Rate of Short- and Long-Term Matings

Because many females experienced multiple matings, we examined whether there might be a pattern with regard to the sequence of ST and LT matings. While nearly half of the females only ever experienced ST matings despite having multiple opportunities to mate during the mating season, just over one third of females had

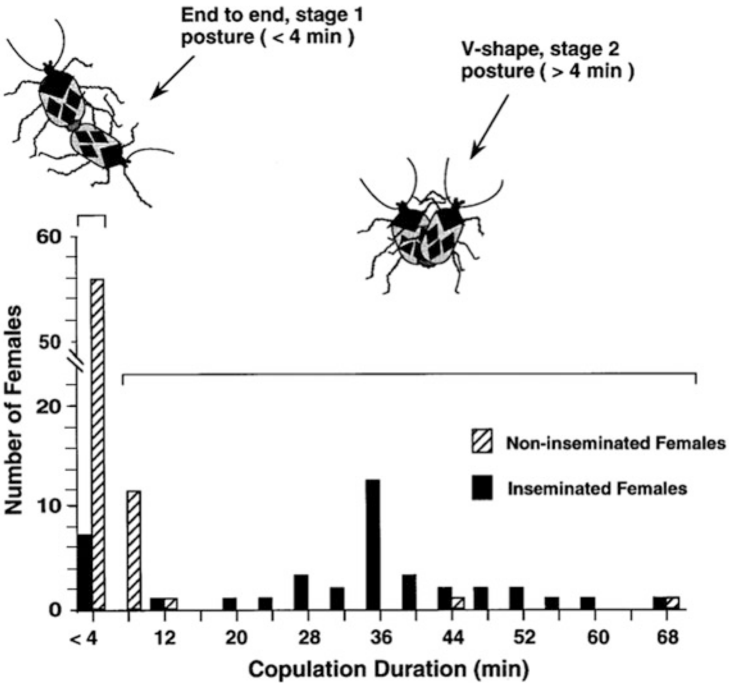

Fig. 3.8 Frequency distribution of duration of long-term-only and short-term-only matings in 1994 and 1995. Stage 1 posture occurred in all ST matings and at the beginning of all LT matings, which then invariably proceeded to stage 2 posture. Adapted from Filippi et al. (2000)

LT matings only (Fig. 3.7b). Among these, one female had two LT matings, and the remainder had only one. Among females that had both ST and LT, roughly equal numbers of females had one or two ST matings before or after the LT mating. Only one female had an ST mating followed by an LT mating and then another ST mating. Among the four groups of females that experienced ST mating at any point, those that had them after experiencing an LT mating had significantly more ST matings. This suggests that LT females might be more attractive to males, who then attempted many more matings that ended up as ST matings.

We examined the duration of mating and insemination rate of females experiencing only ST or LT matings in 1994 and 1995. The frequency of durations was bimodal, with an ST peak at 4 min and an LT peak at 36 min. Only 12% of females that had a copulation duration of less than 12 min had evidence of sperm transfer to their spermatheca, while 94% of females experiencing a copulation of more than 12 min had sperm transfer to their spermatheca (Fig. 3.8). Clearly the LT mating is a far more effective way for males to achieve insemination and is likely to be the actual goal of all males. In many heteropterans, such as lygaeid bugs, the spermathecal duct tends to be long and convoluted, and the prolonged copulations seen in species of this group are apparently related to the time it takes for the male to navigate the intromittent organ to and then through the spermathecal duct to the spermatheca

(Micholitsch and Pass 2000; Himuro and Fujisaki 2015). However, the spermathecal duct in *P. japonensis* is simple and quite short (Pluot-Sigwalt and Lis 2008). Females tend to have only one long-term mating, so the likelihood that males are spending time displacing sperm (Parker 1970; Thornhill and Alcock 1983) also seems unlikely. It might just be that it takes time for the sperm to travel to the spermatheca. Unfortunately, we have not carried out detailed examination of the female reproductive tract at various times during the long-term copulation, which could help answer this question. Another puzzle that has not been resolved is the rhythmic thrusting phase at the beginning of stage 1 in the ST mating. We have stated that there is no courtship behavior prior to intromission, but could the rhythmic thrusting behavior that we assumed was ejaculation actually represent copulatory courtship in the context of cryptic female choice? The behavior certainly meets the criteria of Eberhard (1994, 1997). The jerking motion, similar to what is described for water striders (Arnqvist and Danielsson 1999), is repeated rhythmically, it produces stimulation of the female, it is mechanically "irrelevant" to the physical coupling, and it does not suggest another function. We conclude that this is a strong possibility, but one that requires further scrutiny.

There was considerable variation in the proportion of ST and LT matings from year to year. In 1992, 93% of observed matings were ST and only 7% were LT (Tsukamoto et al. 1994). However, in 1994, among the natural population, 46% were ST and 54% were LT. The respective values for the manipulated matings in 1994 were 67.5% and 32.5%, so the confinement to the mesh cages might have affected the behavior; in 1995 the respective ratios for the manipulated matings were 79% and 21%.

3.10 Effect of Female Body Size, Weight, and Ovarian Development on Mating Duration and Insemination Rate

We examined whether body size, weight, or ovarian development of females impacted the duration of mating and insemination success rate. No correlation was found between female body size and weight and whether she experienced a short- or long-term mating, or no mating at all (Filippi et al. 2000). However, we found evidence that ovarian development affected mating status. We used the criteria for ovarian development described above (Sect. 3.7). In 1995, 45% of control females enclosed on the host tree that did not mate but might have mated if they had been given the opportunity had stage 1 ovarian development (early previtellogenesis). Females that had ST or LT matings were dissected either within 8 h of mating (5% within 30 h) as a proxy for ovarian development at the time of mating or after being allowed to feed for 11–58 days after mating, during which time post-copulation ovarian development could occur. Among the ST individuals soon after mating, 87% had no or the earliest stages of previtellogenesis, and 13% had late previtellogenesis

(Fig. 3.9). None had vitellogenesis. Moreover, among the ST females at the time of mating, only 7% had evidence of sperm transfer. On the other hand, while none of the LT mated females had ovarian development that had advanced to the stage of vitellogenesis at the time of mating, significantly more of them, fully 91% had evidence of sperm transfer. When compared to the control females, there was no significant difference in ovarian development of the ST mated females soon after mating, but the ovarian development of the LT mated females was significantly more advanced than that of the ST mated females (Mann-Whitney U test, $p < 0.03$). Interestingly, after being allowed to feed for a minimum of 11 days after mating, the

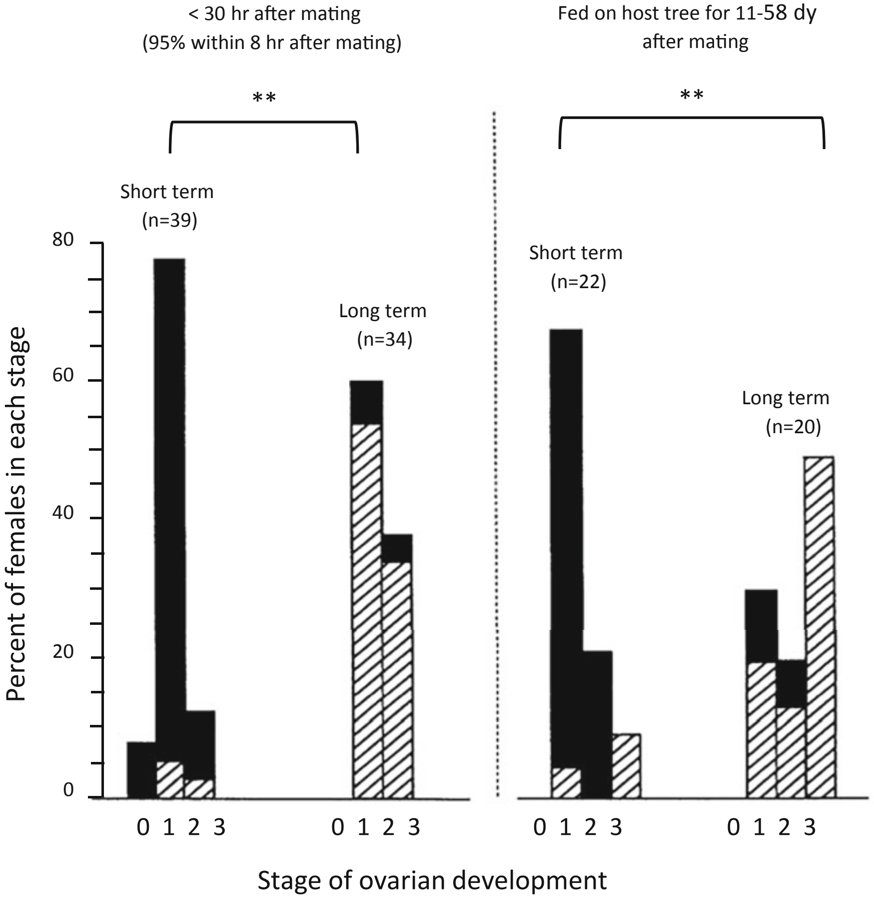

Fig. 3.9 Relationship between stage of ovarian development, mating type (ST, LT), and insemination rate. Hatched areas indicate percent of females that were inseminated. **, Mann-Whitney U test, for differences between ST and LT ovarian development before feeding, $p = 0.005$; for differences between ST and LT ovarian development after feeding, $p = 0.004$. For differences in insemination rate between ST and LT before and after feeding, $p < 0.001$. Adapted from Filippi et al. (2000)

ovarian development of ST mated females increased somewhat, with all females showing some ovarian development and 10% indicating vitellogenesis, but nearly 70% remained in the earliest stage of previtellogenesis. Moreover, only 11% of these females had been inseminated. On the other hand, 62% of females of the LT females had vitellogenesis, and over 85% of these females were inseminated. The findings indicate that females accepting LT matings have greater development of the ovaries at the time of mating and progress to mature ovaries after feeding, while the ovarian development of ST mated females is significantly less. Moreover, LT matings result in significantly greater insemination success. It seems likely that females engaging in LT matings are more receptive because their ovaries are more developed.

3.11 Effect of Male Body Size on Long-Term Mating Success

We also monitored a natural subpopulation of ~3000 individuals in 1994 to determine whether larger males were more successful at achieving LT matings. We individually marked and measured pronotum width and body weight of all males and females 1 month prior to the start of the mating season and returned them to their original site. On each of 7 days during the course of the mating season mating behavior was observed. When a mating was observed, individuals in the mating pairs were noted, and the ratio of female to male body size was calculated. Females are always larger than males, but we predicted that the ratio of female size to male size would be smaller in LT copulations. Interestingly, there were no significant differences in the body size ratios between short- and long-term matings (Filippi et al. 2000). Han et al. (2010, 2018) used the laws of physics, specifically mechanics, to discover that female resistance to mating has led to a size-assortative mating and sexual size dimorphism in water striders. Males of this species use the same grasping pattern across the female dorsum to achieve mating as seen in *P. japonensis*. They found that a particular male-to-female size ratio (0.86) was the optimal ratio for males to effectively grasp the female. Larger and smaller ratios were considerably less successful. We have not examined this possibility in *P. japonensis*, but it would be interesting to explore. Another possibility is that after expending so much energy flying and vigorously repeatedly mating, males might not have the energy to subdue the female. This might be the cue to the female that he has also depleted his sperm on earlier matings, as seen in the stream-dwelling isopod, *Lirceus fontinalis*. Sparkes et al. (2002) found that females resisted all males, but they resisted low-glycogen males more vigorously; successful males had higher levels of glycogen. They also found that females mating with recently mated males had an 18% reduction in fertilization success. With the high rate of matings that male *P. japonensis* engage in and no apparent refractory period in between matings, females would benefit by mating with the most vigorous males, as they would be most likely to have sufficient sperm to fertilize all of their eggs. The data indicate that nearly all females that had long-term matings had only one LT mating, indicating that one LT mating is sufficient to fertilize all of a female's eggs. Thus, females would benefit from mating with a male that is likely to fertilize all of her eggs and refusing all other mating attempts. Females also only lay one egg mass in their lifetime (see Chap. 6), so optimizing fertilization rate of the lone egg mass is critical. Clearly, much work on mating behavior in this species remains to be done!

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Lisa Filippi initiated the work on *Parastrachia japonensis* at Mt. Hinokuma Park in Kanzaki Town, Japan, where she earned her M.S. and doctoral degrees elucidating many of the amazing physiological and behavioral traits of this fascinating insect through field and laboratory studies. She is currently a Professor of Biology at Hofstra University in New York.

Mantaro Hironaka in fact applied to Saga University in order to eventually join the *P. japonensis* research team. He proved to be a brilliant, enthusiastic, energetic, and creative member of the team who contributed significantly to a variety of behavioral studies. Mantaro went on to get his doctoral degree in Dr. Tojo's laboratory from the United Graduate School of Agriculture, Kagoshima University, focusing on the neurological processes involved in the complex navigation that *P. japonensis* uses when provisioning. He is currently an Associate Professor at the Department of Applied Entomology, Faculty of Bioproduction Science, Ishikawa Prefectural University.

Shintaro Nomakuchi an ecologist focusing on insect behavior, came to Saga University in 1993 as an Associate Professor, and was keen to share his very valuable expertise and join the *P. japonensis* research team, which he eventually headed. The work on clarifying the quality of the habitat, the techniques for monitoring the subpopulations and for carrying out a variety of ecological and behavioral studies, as well as the methods for performing robust data analyses, all got a hefty boost, and we were off and running. He is a Professor Emeritus of the Faculty of Agriculture at Saga University.