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Multiple paternity in the invasive spotted lanternfly (Hemiptera: Fulgoridae)

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In biological invasions, multiple paternity can preserve genetic diversity over time and space and contribute to invasion success. Therefore, knowledge on the mating system of invasive species is essential to develop adequate management practices to mitigate their impact on ecosystems. The spotted lanternfly, *Lycorma delicatula* (White, 1845), is an invasive pest that has colonized more than 10 eastern US states in less than 10 yr. Multiple paternity may contribute to its success, but little is known about spotted lanternfly's mating system. We explored the mating system using mated females and female–egg mass pairs sampled in the field. First, we assessed the existence of multiple mating by counting the number of spermatophores in the genital tract of all females. Second, we searched for genetic evidence for multiple paternity within egg masses by genotyping the female–egg mass pairs at 7 microsatellite loci. Third, we assessed whether multiple mating was correlated with female traits and distance from the introduction site. One to 3 spermatophores per female were found during dissections, confirming the existence of polyandrous female spotted lanternfly. We found genetic evidence for a minimum of 2 fathers in 4 egg masses associated with polyandrous females, validating multiple paternity in spotted lanternfly. Multiple paternity was associated with egg mass size, and multiple paternity was highest in populations closest to the original introduction site and decreased toward the invasion front. Multiple paternity may contribute to the invasion success of spotted lanternfly, and control efforts should consider the mating system and the implications of its spatial patterns.

Key words: biological invasion, *Lycorma delicatula*, mating system, microsatellite, polyandry, reproductive system

Introduction

Introduced populations typically retain only a fraction of the genetic variation from their native range due to the limited variation encompassed in the few founding individuals, and genetic drift (Lee 2002, Puillandre et al. 2008). This significant reduction in genetic diversity, a genetic bottleneck, can have detrimental consequences for the long-term survival, through inbreeding depression, and adaptability of introduced populations (Reed and Frankham 2003). In some species with asynchrony between mating and fertilization events, females have the capability to mate multiple times, store sperm from different males, and fertilize their offspring with multiple males (Taylor et al. 2014). Mating systems including polyandrous females play an important role in maintaining genetic diversity from one generation to another (Jennions and Petrie 2000). In biological invasions, multiple paternity can preserve genetic diversity over time and space, avoid inbreeding, increase individual and population fitness, increase effective population sizes, and thus contribute to the invasion success (Miller et al. 2010, Awad et al. 2015). Multiple mating is not necessarily indicative of multiple paternity, however, since females mated multiple times may exert postcopulatory selection that biases paternity in favor of one male (reviewed in Jennions and Petrie 2000; e.g. Loo et al. 2018). By gaining knowledge on the mating systems of invasive species, we can get valuable insights into the success of these populations, and develop adequate management approaches to mitigate their impact on ecosystems.

The spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae, White, 1845), is an invasive pest that has colonized more than 10 states of the eastern United States in less than 10 yr and is responsible for significant damage to the timber and wine industries (Urban 2020, Urban and Leach 2023). Multiple paternity,

by protecting the genetic diversity of this species in its invasive range, could be one of the biological characteristics that contribute to its invasion success and fast progression. During the peak mating season in September–October (Baker et al. 2019), male spotted lanternfly transfer a spermatophore that females store in their spermatheca (Wolfin et al. 2019). Fertilization occurs at the time of egg laying, 30–50 days after the adult emergence (Laveaga et al. 2023), and before all adult spotted lanternfly die off for the winter. Little is known about spotted lanternfly mating systems and whether females mate with multiple males.

Using gravid mated females and female–egg mass pairs collected in the field, we explored the mating system of spotted lanternfly in 3 successive steps. First, we assessed the existence of multiple mating in females through dissections of female genital tract and counting the number of spermatophores stored in the spermatheca. The spermatophore is made of proteinaceous secretions of the male accessory glands that enclose the sperm. Its presence and number are thus indicative of the number of times a female has mated. Second, we genotyped 11 female–egg mass pairs at 7 microsatellite markers to obtain genetic evidence of multiple paternity within egg masses in this species. We determined whether multiple mating resulted in multiple paternity, i.e., whether the number of mates (number of spermatophores) was directly in line with the number of fathers. Third, we tested the correlations between female and population-level traits and mating system. At the individual level, we hypothesized that more fecund females may attract more males and mate multiply, in accordance with the fecundity limitation hypothesis that predicts that the potential gain in fitness for each mating male increases when females have higher numbers of offspring (Avise and Liu 2011, Dobson et al. 2018). At the population level, we hypothesized that if multiple paternity represents an advantage for invasion success, the rate of polyandrous females should increase with distance from the introduction site.

Methods

Sample Collection

Gravid mated females

Spotted lanternflies were collected at 24 locations within the northeastern United States in 2020 (Supplementary Fig. S1A in Supplementary Material 1). Sampling occurred in October 2020 (plus one location sampled in September, Supplementary Table S1), which was near the end of the spotted lanternfly reproductive season when most females were expected to be mated. Individuals were manually collected from 1 to 3 trees per location and killed by freezing on site. They were kept at –20 °C until dissection. Females were distinguished from males in the lab based on the presence of red valvifers on the distal part of the abdomen. From these females, we selected the individuals with swollen abdomens showing large amounts of yellow tissue, which were presumably gravid and in which we would likely find unused spermatophores. This yielded 9–16 gravid females per location for dissections, for a total of 248 females.

Female–egg mass pairs

Females and their respective egg masses were collected between 15 October and 1 November 2021 at 3 locations in eastern Pennsylvania (Supplementary Fig. S1A). Females were observed laying eggs and collection happened, while the female was covering her egg mass with wax to make sure that all eggs had been laid (Supplementary Fig. S1B). Only egg masses clearly separated from other egg masses were collected to avoid mixing eggs from different egg masses. Additionally, some gravid females were collected in the field and kept in the lab in individual containers until they had laid eggs or died (maximum of 5 days) to increase the number of samples (Supplementary Fig. S1C). In total, 42 female–egg mass pairs were collected for analyses of multiple paternity, including 33 pairs collected in the field and 9 obtained in the lab. The average number of eggs per egg mass was 31 ± 12 SD (minimum–maximum: 4–61). The number of eggs per egg mass did not differ between egg masses collected in the field and in the lab (t-test, t = 0.91, df = 40, P = 0.37). All females were preserved in 95% ethanol at –20 °C until DNA extraction. Eggs were incubated for 10–12 wk at room temperature (approx. 20 °C) to allow embryo development to increase the amount of DNA for genetic analyses and then were preserved in 95% ethanol at –20 °C until DNA extraction.

Anatomical Detection of Multiple Mating

The spermatheca of all 290 females were dissected to detect evidence of multiple mating based on 2 criteria: (i) the number of spermatophores and (ii) the number of male genital parts found at the surface of the spermatophores. Each male transfers a single spermatophore into the female's genital tract during mating, so that the number of spermatophores in the spermatheca is indicative of the number of males that mated with the female. Spermatophores are usually of regular, oval shape, but can sometimes be distorted or fragile due to internal body pressure and preservation, making counts more difficult. We found male genitalia, generally in pairs, inside the spermatheca of 77% of gravid mated females that contained a spermatophore (Supplementary Fig. S2). This suggests that there is a natural mutilation of male genitalia during mating since none of our females were forcibly uncoupled from their mates and that these genitalia are transported further in the female oviduct after mating than when the pair is in copula (see Wolfin et al. 2019). In some cases, no male genitalia were found on the spermatophore, or an odd number of male genitalia was found in the spermatheca, suggesting that one or both of them were in the bursa copulatrix below the spermatheca or were not removed during copulation. We considered finding either more than 1 spermatophore or more than 2 male genitalia inside the spermatheca as evidence of multiple mating.

Genetic Detection of Multiple Paternity

The probability of detecting multiple paternity in spotted lanternfly was assessed by 2 complementary methods to determine sample sizes required for genetic analyses. First, we calculated the statistical power at the population level, which is the probability of sampling eggs from different fathers in at least 1 egg mass when sampling egg masses of unknown paternity status within a population, using the method proposed by Veliz et al. (2017). Even though this method has been developed for highly fertile species, it is applicable to species with lower fertility regardless of brood size because it is based on simple sampling probabilities with binomial coefficients to calculate paternity detection probabilities that are effective for any brood size. We advocate for using this method as a power analysis whenever embryos are sampled from a brood, independently of the brood size, because it allows the user to define the number of brood and the number of embryos per brood that optimize the probability of detecting multiple paternity at the population level while controlling costs. Even though we genotyped egg masses showing evidence of multiple mating based on dissections, we set the proportion of egg masses sired by multiple males to 0.5 in this method because multiple mating does not necessarily indicate multiple paternity. We set

the total number of eggs in an egg mass to 31 in accordance with our female–egg mass pair results. We tested scenarios of balanced (50% of embryos sired by male 1, 50% of embryos sired by male 2) or skewed (90% of embryos sired by male 1, 10% of embryos sired by male 2) paternity and considered 4, 6, 8, and 10 eggs sampled per egg mass and 6, 8, and 10 total egg masses. Second, we calculated the probability of detecting multiple paternity within an egg mass sired by 2 males given a number of genetic markers with the PrDM program (Neff and Pitcher 2002). Parameters were set to 4 or 7 loci with 4 alleles of equal frequency each, balanced (0.5) or skewed (0.9) paternity, and 4, 6, 8, or 10 eggs sampled per egg mass. Based on the results from these analyses, we felt confident in testing a minimum of 10 egg masses and 8 eggs per egg mass.

Eleven female–egg mass pairs were genotyped to detect multiple paternity within egg masses. Eight of these 11 were females assumed to have mated multiply based on the presence of 2 or 3 spermatophores during dissections, while the remaining 3 females were assumed to have mated a single time based on the presence of one spermatophore. We genotyped 8–10 embryos per egg mass and all 11 females for a total of 104 individuals. Eggs within egg masses were gently separated with forceps and randomly selected for inclusion in analyses. Genomic DNA was extracted from whole eggs and from female abdominal muscle using the Chelex 10% protocol (Walsh et al. 1991) in 150 µl with 4 µl of proteinase K (20 mg/ml) and lyzed overnight at 56 °C. Proteinase K was inactivated by 15 min at 100 °C.

Embryos and females were genotyped at 8 microsatellite markers in 2 multiplexes using the ABI DS33 dye set (Applied Biosystems, Foster City, CA): Multiplex 1 (MP1) included markers LD-D4, LD-D5, LD-T1, LD-T3 (Park et al. 2013) and Multiplex 2 (MP2) included markers Lde02, Lde07, Lde11, Lde15 (Kim et al. 2011). DNA was amplified using Type-it Microsatellite PCR kits (QIAGEN, Valencia, CA) following the manufacturer's instructions in a 5-µl volume. We used the 3-primer method of Culley et al. (2013), with forward primers tagged with one of the universal primers AP2, Bhg-r, T7 or +19bs and PIG-tailed reverse primers. The PCR mix contained 2.5 µl MasterMix, 0.5 µl 10x primer mix and 2 µl of template DNA. The PCR program was 95 °C for 5 min, 28 cycles of 95 °C for 30 s, 60 °C for 90 s, 72 °C for 30 s, followed by a final elongation of 30 min at 60 °C. PCR products were diluted by 10 and electrophoresed with LIZ500 size standards on an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA) by Genewiz (South Plainfield, NJ). Alleles were scored using Geneious Prime. Lde15 failed to provide consistent peaks and was discarded from the analyses. Nine embryos that were not genotyped at more than 2 loci were discarded, and 3 females could not be genotyped for any MP2 markers. In the remaining individuals, 97% of the genotypes were scored for MP1 and 71% for MP2.

The spotted lanternfly mating system was investigated by 2 complementary approaches: the Gerud 2.0 software (Jones 2005) and a manual allele count. We chose these methods because methods that use maximum-likelihood approaches to infer sibship and parentage, such as the one used in the Colony software (Jones and Wang 2010) tend to overestimate the most likely number of fathers in the case of populations with low genetic diversity like introduced species (Sefc and Koblmüller 2009). Gerud reconstructs the minimum number of potential paternal genotypes and calculates the number of offspring they each sired. We set options to known maternal genotypes and the maximum number of fathers to 3. When multiple paternal genotype combinations were possible, we selected the combination with the highest probability calculated by Gerud based on Mendelian segregation. As Gerud does not accept missing data, we tested 2 alternative datasets: one that maximized the number of eggs (4–8 eggs at 4 loci, "max. eggs") and another that maximized the number of loci (2–7 eggs at 6–7 loci, "max. loci"). As a complementary method that used the full dataset, we also used a manual allele count to determine the minimum number of fathers per egg mass. We assumed that all fathers were heterozygous at all loci. The presence of more than 2 non-maternal alleles in at least 2 loci was considered as evidence of multiple paternity, which allowed for a potential genotyping error or mutation at one locus. In offspring with homozygous genotypes at a locus, or genotypes identical to the mother at a locus, one of the 2 alleles was considered to have been inherited from the father (only admitted cases of allele sharing between male and female). Where the female genotype was missing at a locus, the number of paternal alleles was estimated as the total number of alleles found in the egg mass at this locus, minus 2.

Multiple paternity is expected to contribute to the long-term persistence of populations by maintaining genetic diversity (Jennions and Petrie 2000, Rafajlović et al. 2013). To assess whether multiple paternity may contribute to genetic diversity in spotted lanternfly, we tested whether the allelic richness and expected heterozygosity were higher in egg masses associated with polyandrous females than in egg masses associated with monandrous females with a Mann–Whitney test.

Mating System Correlates

We hypothesized that males may preferentially mate with more fecund females, and as a result, females with egg masses containing more eggs would be more likely to have mated multiply and thus contain more than 1 spermatophore. In invertebrates, female body size often correlates with fecundity (Honěk 1993), and we hypothesized that males could use female body size as a signal of fecundity. We used female wing length as a proxy for body size. The length of the right anterior wing was measured based on photography using ImageJ for 103 mated females: the 42 females from the female–egg pairs and 61 of the other dissected females for which we had high-quality images. Based on the 42 female–egg mass pairs, we tested whether the number of spermatophores per female was related to the number of eggs per egg mass to validate the fecundity–multiple mating relationship. We also tested whether the number of eggs laid was positively linked with female wing length using a linear model to validate the body size–fecundity relationship. Based on 103 dissected females, we then tested whether females with more than 1 spermatophore were larger than females with a single spermatophore using a *t*-test to validate the body size–multiple mating relationship.

We hypothesized that because of the presumed advantages of multiple paternity in colonizing species, the proportion of polyandrous females among all mated females may persist or increase with distance from the introduction point. We tested this hypothesis using a linear model with the average number of spermatophores per female per location as the dependent variable and the distance from the introduction point as the independent variable. We only included population with $n \geq 9$ mated females for a total of 18 populations.

Results

Multiple Mating Detected in Females

Twenty-three of our 248 females did not contain spermatophores or male genitalia and were considered unmated. Although our females were collected over a 7-wk time frame, there was no change in the proportion of mated females over time at sites (Spearman's rank correlation, rho = 0.22, $P = 0.31$, Supplementary

Table S1). All 42 females from the female–egg mass pairs contained spermatophores. Using our criteria for detecting multiple mating in the 290 dissected females, we found evidence of single mating, i.e., a single spermatophore and/or a maximum of 2 male genitalia, in 231 females (87% of mated females); evidence of mating with 2 males, i.e., 2 spermatophores and/or a maximum of 4 male genitalia, in 33 females (12%); and evidence of mating with 3 males, i.e., 2 spermatophores with 5 or 6 male genitalia, in 3 females (1%).

Multiple Paternity Detected Within Egg Masses

The statistical power at the population level was greater than 0.97 in all cases for the balanced paternity scenario (Supplementary Fig. S3A). It was greater than 0.68 and reached 0.89 starting at 6 eggs × 8 egg masses for the skewed paternity scenarios (Supplementary Fig. S3A). The probability of detecting multiple paternity within an egg mass with balanced paternity from 2 fathers was greater than 0.80 above 6 eggs and 4 loci (Supplementary Fig. S3B). For an egg mass with skewed paternity, it was greater than 0.5 with 8 eggs and 7 loci, or 10 eggs and 4 loci (Supplementary Fig. S3B). These simulations indicate a strong power to detect multiple paternity in case of balanced paternity, and a moderate power in case of skewed paternity (1 in 10 offspring sired by male 2) when considering 4–7 genetic markers with 4 equal alleles, 8 egg masses, and 4–10 eggs per egg mass.

We observed 3–5 alleles per microsatellite marker. All embryo genotypes were compatible with the maternal genotypes, i.e., each egg had one common allele per loci with the corresponding female.

Gerud (maximum eggs and maximum loci datasets) and the manual allele count detected single paternity within the egg masses associated with the 3 presumed monandrous females (JF12, JF13, JF14, Table 1). Gerud (maximum loci dataset) and the manual allele count found evidence for 2 fathers for 4 of the 8 egg masses associated with presumed polyandrous females (JF4, JF8, JF22, JF3) and for 1 father for the other 4 egg masses (JF7, JF9, JF16, JF20). The other Gerud dataset (maximum eggs) found similar results but detected a single father in JF22. For egg masses with multiple paternity, Gerud estimated the proportion of eggs sired by the first male between 50% and 75% (Table 1).

Egg masses from polyandrous females did not have a higher allelic richness ($W = 6$, $P = 0.28$, Fig. 1A) or a higher expected heterozygosity ($W = 4$, $P = 0.13$, Fig. 1B) than egg masses from monandrous females.

Mating System Correlates

Egg masses from females with multiple spermatophores contained more eggs than egg masses from females with a single spermatophore, validating the fecundity–multiple mating relationship ($t = -2.8471$, df = 40, $P < 0.01$, Fig. 2A). The number of eggs per egg mass was not correlated with female wing length ($P = 0.1$, Fig. 2B), rejecting the body size–fecundity relationship. Females containing more than one spermatophore were not larger than females containing a single spermatophore ($t = -0.51$, df = 101, $P = 0.61$, Fig. 2C), rejecting the body size–multiple mating relationship. The average number of spermatophores per mated female was negatively correlated to the distance of the sampling site from the introduction site ($P = 0.04$, Fig. 2D; Supplementary Table S1).

Discussion

We provided the first evidence of polyandrous spotted lanternfly females, based on anatomic evidence that females mated with 1–3 males each. Genetic analyses demonstrated that multiple mating in spotted lanternfly resulted in multiple paternity within some egg masses. Multiple paternity is ubiquitous and common in nature, although its prevalence is variable within and across species (Taylor et al. 2014), and it has been genetically demonstrated in a limited number of nonsocial insect species (e.g., Song et al. 2007, Haddrill et al. 2008, Seabra et al. 2013).

The link between multiple mating and multiple paternity was not systematic across our samples. The 7 microsatellite markers showed low allelic richness, which complicates parentage analyses, even with high sample sizes. The number of fathers we identified by Gerud and a manual allele count is conservative because it

Table 1. Number of fathers estimated per egg mass through dissection and genetic analysis (Gerud and manual allele count). Sample sizes vary because Gerud does not accept individuals with missing genotypes. NA = Gerud could not generate results due to a high number of potential parental genotypes

		Estimated number of fathers (eggs sired by each male)				
			Gerud			
Female–egg mass pair code	Number of eggs genotyped at >2 loci (proportion of egg mass)	Female dissection	Max. eggs (4 loci)	Max. loci (6–7 loci)	Manual allele count	Match between anatomy and genetics
JF12	8 (20%)	1	1 (4)	1 (3)	1	Yes
JF13	7 (23%)	1	1 (6)	1 (2)	1	Yes
JF14	6 (17%)	1	1 (5)	1 (2)	1	Yes
JF4	8 (17%)	2	2 (4/4)	2 (4/3)	2	Yes
JF7	9 (15%)	2	1 (8)	1 (7)	1	No
JF8	8 (19%)	2	2 (4/2)	2 (2/3)	2	Yes
JF9	7 (23%)	2	1 (7)	1 (5)	1	No
JF16	8 (15%)	2	1 (5)	1 (3)	1	No
JF20	8 (24%)	2	1 (8)	NA	1	No
JF22	7 (18%)	2	1 (7)	2 (3/3)	2	Partial
JF3	8 (25%)	3	2 (4/2)	2 (3/1)	2	Partial

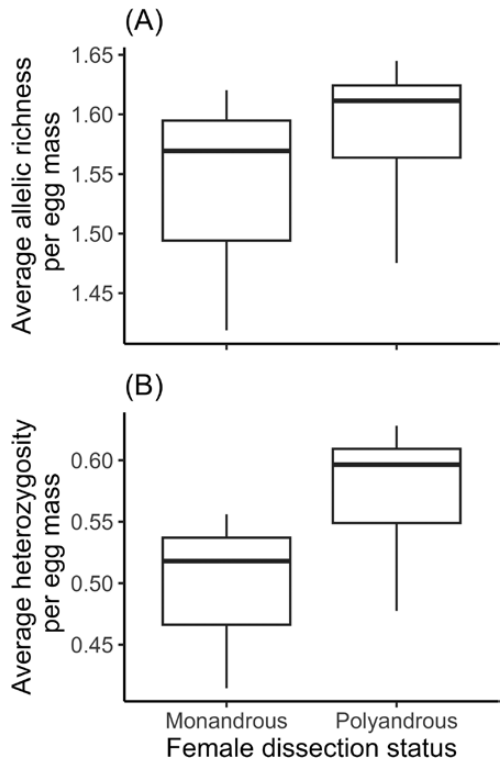

Fig. 1. Comparison of the genetic diversity of egg masses from polyandrous and monandrous females. A) Allelic richness, B) expected heterozygosity.

cannot be overestimated, but it can be underestimated, especially when allelic richness is low. Even though microsatellite markers are standard for parentage analysis (Taylor et al. 2014), markers with more variability are needed to confirm whether polyandry systematically results in multiple paternity in spotted lanternfly. Based on our calculated probabilities of detection of multiple paternity, it is possible that multiple paternity went undetected in egg masses from some polyandrous females because of highly skewed paternity ratios compared to sample sizes. Alternatively, polyandry may not always result in multiple paternity, i.e., the number of mates may be higher than the number of fathers. Such difference may stem from postcopulatory selection by females, known as cryptic female choice that biases paternity toward the best male (reviewed in Jennions and Petrie 2000). This would be a rich area for future work to explore.

Our hypothesis that more fecund females, i.e., females with larger egg masses, would be more likely to mate multiple times was supported. This result is in line with the fecundity limitation hypothesis (Avise and Liu 2011), which predicts that multiple paternity should be correlated with brood size since larger broods give males more of a chance to sire offspring than smaller ones when mating multiply.

This finding has important consequences, as a few multiply mated females introduced in a new area could potentially lay a large number of eggs carrying important genetic variation. This could overcome the genetic and demographic bottleneck sometimes observed during the initial phases of biological invasions, and explain the invasion success of spotted lanternfly (see explanations for apparent genetic paradox in Estoup et al. 2016). Even though mated females do not fly as spontaneously as non-mated females (Wolfin et al. 2019), multiply mated females would constitute a tangible threat if they or their egg masses were passively transported to noninvaded areas. Management strategies focusing on limiting the transportation of spotted lanternfly are thus especially necessary during the reproductive season.

Going forward, it is necessary to identify which traits may serve as proxies for female fecundity. Body size, as represented by wing size, may not be a good proxy for fecundity in spotted lanternfly, as the body size–fecundity and the body size–multiple mating hypotheses were both rejected by our data. However, wing size may not be the best proxy for body size and other traits may better represent body size. Morphological traits correlated with female fecundity (e.g., dry body weight, abdominal area), traits that make more fecund females mate multiply or traits that males use to detect more fecund females also need to be explored. Additionally, we observed that a fraction of females showing abdominal yellow were unmated, in line with observations from Wolfin et al. (2019). We also noted that some mated spotted lanternfly females had a brown patch above the valvifers that unmated spotted lanternfly females do not have and that it differs from the wax residues found on the valvifers after egg laying. It would be interesting to formally quantify whether a large area of abdominal yellow and the presence of a brown patch above the valvifers are indicators of mated females, so the mating status of a female could be detected based on external morphology only.

Interestingly, the prevalence of multiple mating decreased with distance from the introduction point (Fig. 2D), contrary to our hypothesis that it should increase due to its evolutionary advantages (Miller et al. 2010, Taylor et al. 2014). The occurrence of multiple mating may be linked with population characteristics, notably male availability and population density, which may both be limited in newly colonized areas. Indeed, we found widespread evidence for male genitalia in the spermatheca of mated females. Description of the male external anatomy in spotted lanternfly is scarce, and we tentatively identify them as genital claspers, also called genital styles or gonoforceps, based on similarity across the Hemiptera order (Marks 1951). The importance of genital claspers in intromission is described in a Heteroptera (Moreno-García and Cordero 2008), which is compatible with these parts being found in the female after mating. Abscission of male genitalia is known to reduce sperm competition when it plugs the female oviduct and is often associated with male sacrifice behavior (cannibalism or spontaneous death after copulation) in spiders (Miller 2007, Uhl et al. 2010). In the case of spotted lanternfly, male genital mutilation does not prevent multiple mating in females, and whether it causes male monogyny (males mating with a single female) or death must be determined in future studies. Regardless, a local deficit in males would likely cause lower rates of multiple paternity. The only other known geographic pattern of variation in polyandry is a weak positive correlation noted between levels of polyandry and latitude across all taxonomic groups that occurs at a much larger scale than our study (Taylor et al. 2014).

Our work demonstrates that the spotted lanternfly mating system consists of variable proportions of monandrous and polyandrous females, and we suggest further research exploring monogynous males. Additional work is required to determine whether dissection alone could be used to detect multiple paternity, which would help investigating the variation in mating systems in a range of populations. Multiple paternity within egg masses, clutches, or litters has benefits beyond the individual level (Jennions and Petrie 2000). Although we could not demonstrate the benefits of multiple paternity on allelic richness or heterozygosity with our sample sizes, multiple paternity constitutes an advantage for species at the population level as it preserves genetic diversity from one generation to another, which is especially beneficial in biological invasions that are affected by strong founder effects when introduced in a new area (Miller et al. 2010, Yue et al. 2010). The genetic diversity that can be carried by a single mated female introduced in an uninvaded area is thus very important for the success of an invader.

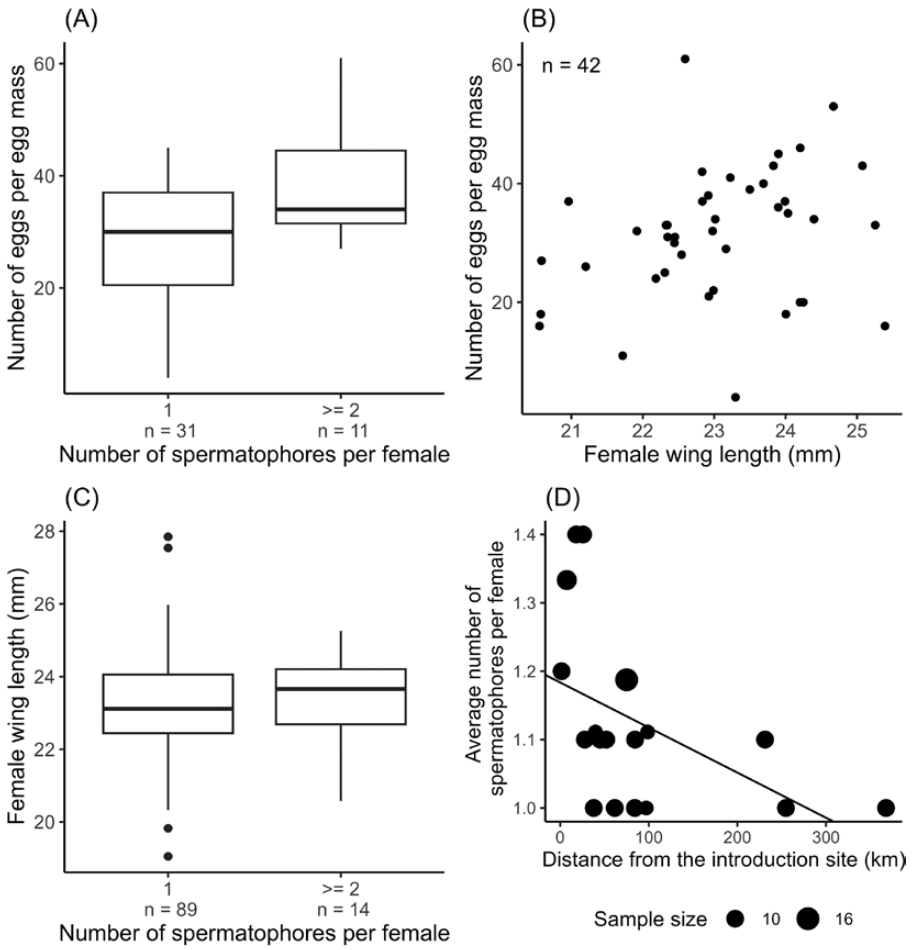

Fig. 2. Traits correlated to multiple paternity. A) Fecundity–multiple mating relationship. B) Fecundity–body size relationship. C) Body size–multiple mating relationship. D) Decrease in the occurrence of multiple paternity with distance from the introduction site.

Finally, knowledge on mating systems is essential to identify adequate management techniques. For example, the release of sterile males (or sterile insect technique), an effective control measure in insect pests (Teem et al. 2020), would have very limited success in polyandrous species because females would still be able to mate with nonsterile males and produce offspring. In addition, knowing the mating system allows managers to identify periods at increased risk of establishment of new populations due to polyandrous females. This is especially important if spatial variation in the mating system correlates with the direction of invasion, as we have shown here. Often in invasions, management efforts are focused on the invasion front to understandably slow the spread of the invasion. However, if populations near the invasion core are polyandrous, they may maintain genetic diversity that can proliferate through the invaded range and improve the invasion success at the front. This provides further evidence that different management strategies may be needed at the invasion core and front to slow the invasion as has been previously suggested (Ramirez et al. 2023).

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Author Contributions

Nadège Belouard (Conceptualization [Equal], Formal analysis [Lead], Investigation [Lead], Visualization [Lead], Writing – original draft [Lead], Writing – review & editing [Equal]), and Jocelyn Behm (Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Lead], Investigation [Supporting], Resources [Lead], Visualization [Supporting], Writing – review & editing [Equal])

Supplementary Material

Supplementary material is available at *Environmental Entomology* online.

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