

High promiscuity among females of the invasive pest species *Drosophila suzukii*

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Abstract

Drosophila suzukii (Matsumura, 1931), the spotted-wing drosophila, is a highly invasive fruit fly that spread from Southern Asia across most regions of Asia and, in the last 15 years, has invaded Europe and the Americas. It is an economically important pest of small fruits such as berries and stone fruits. *Drosophila suzukii* speciated by adapting to cooler, mountainous, and forest environments. In temperate regions, it evolved seasonal polyphenism traits which enhanced its survival during stressful winter population bottlenecks. Consequently, in these temperate regions, the populations undergo seasonal reproductive dynamics. Despite its economic importance, no data are available on the behavioural reproductive strategies of this fly. The presence of polyandry, for example, has not been determined despite the important role it might play in the reproductive dynamics of populations. We explored the presence of polyandry in an established population in Trentino, a region in northern Italy. In this area, *D. suzukii* overcomes the winter bottleneck and undergoes a seasonal reproductive fluctuation. We observed a high remating frequency in females during the late spring demographic explosion that led to the abundant summer population. The presence of a high degree of polyandry and shared paternity associated with the post-winter population increase raises the question of the possible evolutionary adaptive role of this reproductive behaviour in *D. suzukii*.

KEYWORDS

adaptation, *Drosophila suzukii*, pest insect, polyandry, remating

Simone Puppato and Giulia Fiorenza contributed equally to this study.

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1 | INTRODUCTION

Drosophila suzukii, the Spotted Wing *Drosophila*, is a highly invasive fruit fly that appeared on the Western fruit crop scene more than a decade ago (EPPO, 2023). Indeed, it has become an economically important worldwide pest of berries and stone fruits, including apple, apricot, blackberry, blueberry, cherry, fig, grape, kiwi, mango, persimmon, plum, raspberry, strawberry and sweet orange (Asplen et al., 2015; EPPO, 2023; Grassi et al., 2018).

Originating in Southern Asia, *D. suzukii* was reported across several regions of Asia during the second half of the 20th century and invaded parts of the European and Mediterranean regions and the Americas over the last 15 years (EPPO, 2023).

Within the *melanogaster* group, it represents a striking case of ecological, physiological and behavioural adaptations, and genetic innovation. These traits, together with its high reproductive potential, give this species a plasticity that is a key to its success (Little et al., 2020; Shearer et al., 2016; Tochen et al., 2014).

It is thought to have diversified in the Miocene (Tortonian) simultaneously with the uplift of the Himalayan/Tibetan plateau, an intensification of the monsoon cycles, and lower temperatures (Ometto et al., 2013). This, together with its current endemic distribution, suggests that *D. suzukii* speciated as a consequence of its adaptation to more temperate, mountainous and forest environments (Markow & O'Grady, 2005; Ometto et al., 2013).

Unlike most other closely related *Drosophila* species that oviposit on rotting substrates, *D. suzukii* has evolved an oviposition preference for ripening and healthy ripe fruits, resulting in economically important agricultural losses. This is a consequence of an evolutionary shift in its gustatory receptor repertoire with the loss of 20% of the bitter-sensing sensilla in the labellum (Dweck et al., 2021). Thus, egg laying is not deterred by the presence of the bitter compounds that are present in the early stages of fruit maturation (Dweck et al., 2021; Karageorgi et al., 2017; Kee-sey et al., 2015). Compared to *Drosophila melanogaster* (Meigen, 1830), the females evolved a long and serrated ovipositor that facilitated oviposition in a wide range of soft-skinned fruits (Green et al., 2019). Importantly, in temperate regions, *D. suzukii* displays a seasonal polyphenism in morphological, physiological, and behavioural traits that enhance its survival under a range of stressful conditions (Enriquez et al., 2018; Shearer et al., 2016; Wallingford & Loeb, 2016). This polyphenism manifests in two discrete morphotypes in adult flies. Juveniles developing in autumn at relatively low temperatures (10–15°C) and short photoperiod emerge from the pupae as “winter morph” adults. They are characterized by darker pigmentation and longer wings, compared to the “summer morphs” that emerge after the winter bottleneck in the spring to give rise to the new and abundant summer populations (Everman et al., 2018; Fraimout et al., 2018; Grassi et al., 2018; Panel et al., 2020; Shearer et al., 2016; Stephens et al., 2015; Toxopeus et al., 2016; Wallingford et al., 2016). The morphotypes differ in key life history traits, gene expression and metabolic profiles, reflecting their different propensities for cold tolerance versus

reproduction (Panel et al., 2020; Shearer et al., 2016). Consequently, in temperate regions, *D. suzukii* populations undergo seasonal morphological, physiological, and reproductive dynamics with a severe winter population bottleneck. Life history analyses of this fly in laboratory and wild conditions provided basic information on the adaptability of populations to novel environments (Papanastasiou et al., 2021; Tochen et al., 2014). But despite its economic importance, no data are available on the behavioural aspects of the reproductive strategies of this fly. Whether *D. suzukii* displays polyandry is an unexplored aspect of its reproductive biology that deserves attention also in the light of the seasonal reproductive dynamics that its populations encounter. Indeed, polyandry helps populations maintain genetic variability and increases the effective population size, increasing the long-term stability of populations and their reproductive potential (Holman & Kokko, 2013; Lewis et al., 2020). Importantly, it has been suggested that a large effective population size facilitates the speed and strength of adaptation in *D. suzukii* populations (Olazuaga et al., 2022).

Given this background, we explored the presence of polyandry in an established population in a North Italian region (Trentino-Süd Tirol), the first Italian area to be colonized by *D. suzukii* in 2009. In this area, *D. suzukii* can overcome the winter bottleneck and undergo a reproductive seasonal fluctuation (Grassi et al., 2018). We observed a high remating frequency in females of the population during the late spring demographic explosion that led to the abundant summer population. As the post-winter population expansion was found to be associated with high levels of polyandry and shared paternity, this raises questions about the possible evolutionary adaptive significance of this reproductive behaviour in *D. suzukii*. It may, in the long term, reinforce the size and genetic variability of the summer population.

2 | MATERIALS AND METHODS

2.1 | Study site and population density monitoring

The target area was Costasavina, Valsugana, Trento province, Italy (46°03'05" N 11°13'13" E, 500m altitude) (Figure 1), a locality with abundant wild and cultivated hosts across the seasons. Consequently, the locality can support *D. suzukii* populations throughout the year, although with a severe winter bottleneck from December to January (Grassi et al., 2018). In the Costasavina area, *D. suzukii* displays a typical seasonal polyphenism (Grassi et al., 2018). Overwintering morphs are present until April, and these females may contain sperm in their spermathecae stored from autumn matings (Grassi et al., 2018). From the beginning of April, while the males are sub-fertile, the females undergo gradual reproductive maturation together with an increase in the presence of sperm in the spermathecae, which reaches its highest levels from May onwards. Consequently, the females, once fully recovered from overwinter reproductive dormancy, start to oviposit fertilized eggs on wild hosts without requiring further mating (Grassi et al., 2018; Panel



FIGURE 1 Map of Trentino Province, North Italy, indicating the sampling site, Costasavina.

et al., 2018, 2020). This leads to the first appearance of the new generation of the summer morphotype in trap catches in mid-May/June.

Given this background, starting from mid-May to the beginning of August 2019, the dynamics of the population density were monitored by fly trapping and measuring the sweet cherry (*Prunus avium* Linnaeus, 1755) fruit infestation rate. The sweet cherry is a very abundant, unmanaged host in the area.

Twelve monitoring points were set up over two plots separated by 1.5 km. Fly traps were positioned within 2.5 m of unmanaged sweet cherry trees. Each trap (Droso Trap®, Biobest) was filled with 200 mL of Droskidrink® liquid bait (Prantil - 75% apple cider vinegar, 25% red wine and 20 g/L unrefined cane sugar) and a drop of non-ionic surfactant Triton™X-100 (Sigma-Aldrich/Merck Life Science Srl). Traps were refreshed every 7 days and the content taken to the laboratory for inspection. Total catches of *D. suzukii* were recorded each week.

The fruit infestation rate was evaluated weekly from samples of 40–50 cherries randomly collected from trees at each monitoring point and checked under magnification for the presence of the egg's white breathing spiracles protruding out of the oviposition hole on the fruit's surface. The infestation rate was estimated as the percentage of fruits in each sample that contained at least one egg: IR = number of infested fruits / (total number of inspected fruits × 100).

2.2 | Polyandry assessment

The presence and degree of polyandry in the Costasavina population were estimated in June 2019 (week 26), during the exponential phase of the population, as determined by monitoring data derived from weekly trap catches and from the infestation rate. Thirty-one females were collected by aspiration from ripe cherries from several unmanaged *P. avium* trees during two sampling sessions over the course of week 26 (24th and 26th June) and were immediately transferred into individual vials containing water-soaked cotton wool and

an uninfested fresh blueberry (*Vaccinium myrtillus* Linnaeus, 1753) as oviposition substrate. All females were kept in a climatic chamber set to $22 \pm 1^\circ\text{C}$ and $70 \pm 10\%$ RH. Blueberries were provided by a local grower cooperative (Sant'Orsola S.C.A.) and inspected under magnification to assess their uninfested status (absence of *D. suzukii* eggs) before use.

The blueberries and cotton wool were replaced every 48–72 h until the death of the female. The removed fruits were inspected under magnification to assess the number of eggs laid by each female. The blueberries were incubated in new vials to permit the eggs to develop into adults. Offspring from each female were collected every 24 h and sexed. The females and their offspring were frozen and preserved in absolute ethanol until subsequent molecular analyses.

2.3 | Microsatellite (SSRs) markers

Genomic DNA was extracted from whole females and their progeny using a standard protocol (Baruffi et al., 1995). DNA quality and quantity were assessed using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc.). Multilocus genotyping was performed using four dinucleotide repeat microsatellite loci: DS05, DS09, DS15 and DS22 chosen from the previously characterized SSR sequences (Framout et al., 2015; Tait et al., 2017) (Table S1). SSRs are appropriate markers for parentage analyses (Jones et al., 2010).

Amplifications were performed in 20 μL reaction volumes containing 1× PCR Buffer, 0.2 mM dNTP mix, 1.5 mM MgCl_2 , 0.5 μM of each primer, 0.5 U Taq polymerase (Invitrogen) and 1–5 ng of genomic DNA. Amplifications were performed using a Mastercycler Nexus Gradient (Eppendorf) with the following conditions: 94°C for 3 min, 30 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. Amplification products were electrophoresed on 1.5% agarose gels and visualized by exposure to UV light after ethidium bromide staining.

Diluted PCR products were genotyped using an ABI PRISM 310 Genetic Analyser (Applied Biosystems) with GeneScan 500 ROX (Thermo Fisher Scientific) as an internal size standard. Allele sizes were scored using GeneMapper software (Applied Biosystems). An individual was declared null (non-amplifying allele) after at least two amplification failures.

The suitability of the four SSR loci for the assessment of remating was tested on an additional sample of 40 males and females collected in Costasavina on week 26 (June 2019). For each locus, the number of alleles (N_a), their frequencies, observed heterozygosity (H_o) and expected heterozygosity (H_e) were estimated using the program Genepop version 4 (Rousset, 2008). The same software was also used to test for linkage disequilibrium between the loci (100 batches, 1000 interactions per batch) and for deviations from Hardy-Weinberg equilibrium, using Fisher's exact test. The average exclusion probability (Excl.), i.e., the probability of excluding a single unrelated candidate parent from the parentage of a given offspring, knowing the genotype of the second parent, was estimated using the program Cervus 3.0 (Kalinowski et al., 2007). For each locus, the frequency of null alleles was calculated using the Brookfield estimation (Brookfield, 1996) in MicroChecker 2.2.3 (van Oosterhout et al., 2004).

2.4 | Detecting remating

Parentage analysis was performed using different approaches: a Bayesian computation method using SCARE (Jones & Clark, 2003) and a parsimony approach using GERUD2.0 (Jones, 2005).

SCARE was used to determine the remating parameter α , related to the number of different matings per female, and the sperm displacement parameter β . SCARE uses a model (Harshman & Clark, 1998) for multiple mating and sperm displacement within a Bayesian framework that uses Markov chain Monte Carlo to examine the posterior distribution of the model parameters. The mean number of fathers per brood was deduced from the parameter α using the formula $M = \alpha / (1 - \exp\{-\alpha\})$ (Jones & Clark, 2003).

GERUD2.0 uses an exhaustive algorithm to reconstruct parental genotypes from multilocus genotyping data of a progeny array. When the genotype of each brood's mother is known, the analysis is focused on the estimation of the minimum number of fathers per family. If multiple minimum-father solutions are possible, the software ranks the most likely solutions based on Mendelian segregation and allele frequencies in the population. The reliability of the paternity analysis to detect remating was explored with GERUDsim2.0 (Jones, 2005), simulating sets of five replicates using population allele frequencies based on genotyping. Each set of simulations was performed using four SSR loci and 10, 20, 30 or 50 offspring, varying both sperm displacement from 0 to 0.9 (Jones & Clark, 2003) and the number of fathers from two to five. Although GERUDsim2.0 may simulate up to six fathers, given the extremely long computation times required, this option was excluded from our simulations. For the simulations involving 50 offspring, the scenario with five fathers was omitted for the same reason. Each simulation replicate consisted of 1000 iterations, except for the

replicates involving five fathers where the number of iterations was reduced to 100 due to computational limitations.

3 | RESULTS

The results of weekly monitoring of *D.suzukii* adults in Costasavina performed from mid-May to August clearly illustrate the exponential increase in population size from late spring to summer. Indeed, in mid-May, 0.6 ± 0.2 males and 1.8 ± 0.5 females/trap were captured, while 447.6 ± 105.4 males and 215.2 ± 53.6 females were recovered per trap at the beginning of August, with a 275-fold increase in the population density. This population expansion is associated with a high level of variability: a June sample displayed an expected heterozygosity (H_e) of 0.79 and an effective number of alleles (N_e) of 4.76. (Table S2).

Monitoring of cherry fruit showed an increasing trend of infestation, which paralleled the fly population explosion, with $1.7 \pm 1.1\%$ of fruit infested in mid-June, $91.0 \pm 4.6\%$ at the end of June and 100% infestation in early July.

3.1 | Observed remating frequency during the exponential density increase of the Costasavina population

When tested on a sample of 40 males and females collected in Costasavina during the late spring exponential population increase, the four SSRs appeared to be very informative markers for the assessment of remating. They were very polymorphic with a mean PIC estimate of 0.739, the null allele frequencies were lower than the threshold set to 0.05 (Kalinowski et al., 2007) and they provided a very high probability of .966 of excluding an unrelated candidate parent from the parentage when the genotype of the mother is known. Consequently, they were used for parentage analyses on the progeny of females collected in the last week of June. Of the 31 sampled females, 26 produced progenies with an average of 48.7 ± 3.2 eggs and 34.0 ± 2.4 offspring per female across all the oviposition days. Overall, the sex-ratio of the progeny was 1:0.995 (males:females). The mean longevity of the captured females was 33.9 ± 1.3 days. Significant correlations were observed between female longevity, egg production and the number of progeny (Pearson's correlation coefficient, $r = .396$, $p = .045$, and $r = .457$, $p = .019$, respectively). As the total brood size of the 26 families was variable, 12 families distributed among different brood sizes (dark bars in Figure S1) were selected for paternity assays. From each of these families, 50%–100% of the progeny collected on each oviposition day were genotyped, choosing randomly from both sexes. Consequently, 12 mothers and their broods, for a total of 328 offspring (mean per family = 27.3 ± 4.4 SE), were genotyped at the 4 SSR loci and used for the SCARE and GERUD2.0 analyses.

SCARE estimated the parameter α of 4.53 (3.47–5.61, 90% confidence interval) among the wild Costasavina females, which

corresponds to a mean of 4.59 (3.58–5.63, 90% CI) fathers. SCARE also revealed a low sperm displacement β parameter estimated at .33 (0.26–0.40, 90% CI) (Figure 2).

GERUD2.0 detected multiple paternity in 10 of the 12 families (83%), with from two to five fathers detected per brood and a minimum average number of 3.25 ± 0.46 (SE) matings per female (Table 1). GERUD2.0 was also able to reconstruct the relative contributions (in terms of numbers of progeny) of the different fathers in each of the 12 families. Figure 3 clearly shows that the different males had contributed to a significant degree to the progeny of the 10 remated females. The allelic compositions of the different families are quite heterogeneous, depending on the allelic contributions of the different fathers to the broods (Table S3).

GERUDsim2.0 was used to validate the ability of GERUD2.0 to correctly assign the number of fathers, considering the effects of the levels of sperm displacement and brood size. Given that the mean genotyped brood size was almost 30 (27.3 ± 4.4 SE), GERUD2.0 should confidently identify up to four fathers with β of up to .4, whereas the confidence of detecting five fathers drops from around 65% with no sperm displacement ($\beta=0$) to just under 50% with a β of .3. With the sperm displacement value ($\beta=.33$) estimated by SCARE, GERUD2.0, considering 30 progeny, should correctly identify 2, 3, 4 and 5 fathers with 99%, 96%, 83%, and 50% certainty, respectively (Figure 4). These values indicate confidence in identifying the correct number of fathers; otherwise GERUD2.0 will underestimate the number of fathers.

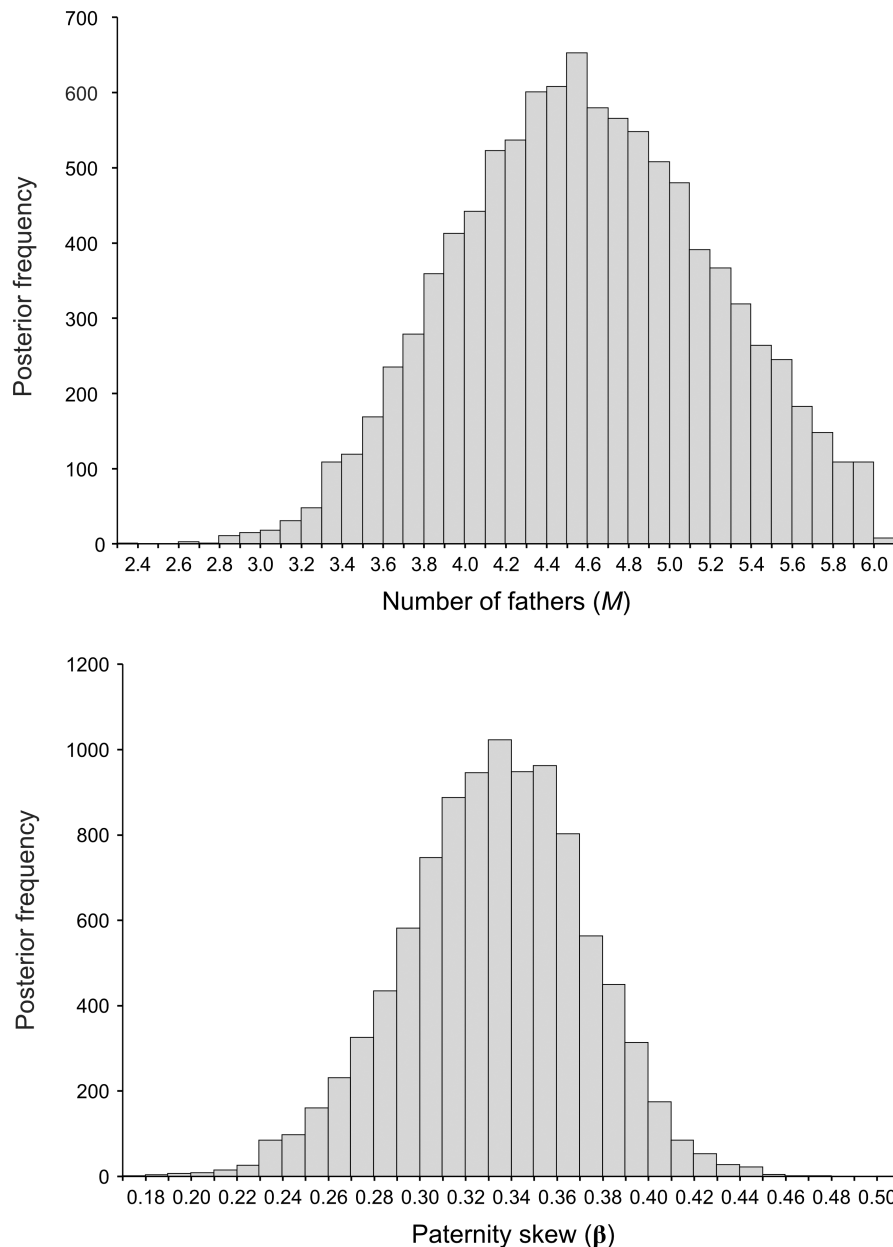


FIGURE 2 Histograms of the posterior frequencies of the number of fathers (M) and the sperm displacement parameter (β) based on the SCARE analysis.

TABLE 1 GERUD2.0 paternity analysis inferred on 12 families from captured *Drosophila suzukii* females.

Family	Offspring	Genotyped offspring	Minimum no. of fathers	Likelihood of the most probable solution	Exclusion probability ^a
U3	38	20	5	4.172E-44	.92
U11	39	20	2	7.918E-14	.67
U12	32	18	2	9.862E-14	.83
U14	14	8	3	3.604E-19	.86
U16	56	56	4	8.416E-50	.91
U17	37	36	4	1.985E-44	.88
U20	36	36	5	1.289E-41	.94
U23	21	21	1	. ^b	.82
U24	21	12	2	. ^b	.84
U25	23	22	1	. ^b	.92
U26	56	54	5	2.459E-48	.88
U28	39	25	5	1.812E-43	.93

Note: The size, number of genotyped offspring, minimum number of fathers, likelihood and exclusion probabilities are indicated for each family.

^aExclusion probability at all loci.

^bUnique solution.

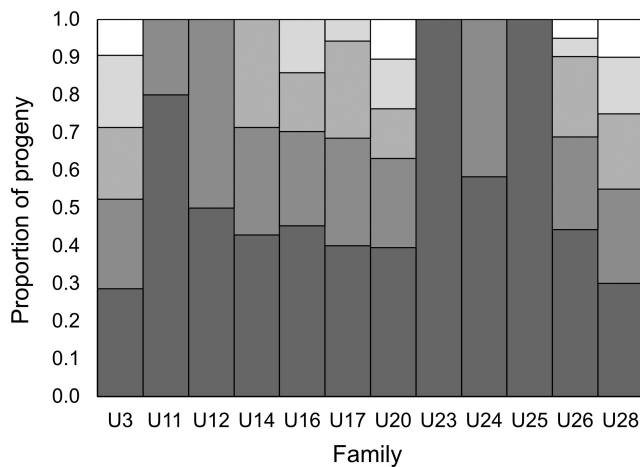


FIGURE 3 Contributions of different fathers in terms of the number of progenies sired in the 12 families as reconstructed by GERUD2.0. The different shaded vertical bars represent the proportion of progeny sired by a given male.

4 | DISCUSSION

A high remating frequency was observed in females in the Costasavina population that, starting in late spring, underwent an exponential increase in density. Moreover, and importantly, the females use the sperm from different males to sire their progeny, which displays high shared paternity.

4.1 | The spring population emerging from the winter bottleneck undergoes a demographic explosion

In the Costasavina area, the first appearance of the new generation of the summer morphotype in trap catches is detected in

mid-May/June (Grassi et al., 2018). From this period, due to the availability of the sweet cherry, *D. suzukii* changes its foraging and oviposition behaviour to favour this highly nutritive host fruit (Little et al., 2020), and the population begins its demographic increase. Indeed, in 11 weeks of monitoring, from mid-May to August (week 31), we observed a population density increase of 275-fold with a sharp increase in the infestation rate of sweet cherry from mid-June to early July.

4.2 | The demographic explosion is associated with a high degree of polyandry and shared paternity

Females collected in late June during the rapid population growth were highly polyandrous, with an average of more than three matings per female (3.25 and 4.59 according to the parsimony approach of GERUD2.0 and the Bayesian SCARE, respectively). As these estimates are derived from paternity tests and are conservative, the actual number of matings per female may be greater.

Importantly, in our sample of 12 families, despite the different brood sizes, we found that the different males contributed significantly to the progeny, which, consequently, were genetically very heterogeneous (H_E from 0.74 to 0.50) at the four SSR loci. These data, together with the fact that we found a low sperm displacement parameter, β , of only .33, indicate the strength of the impact of polyandry on the variability of the population during its demographic increase. Polyandry, associated with the observed high intrinsic rate of *D. suzukii*, may help to buffer the inbreeding effect due to the winter bottleneck, allowing the long-term stability and variability of the summer population. It is noteworthy that *D. suzukii* females continue to oviposit until an advanced age (Papanastasiou et al., 2021). Polyandry also hedges against the subfertility of males that emerge from the winter bottleneck (Grassi et al., 2018; Panel et al., 2020; Papanastasiou et al., 2021).

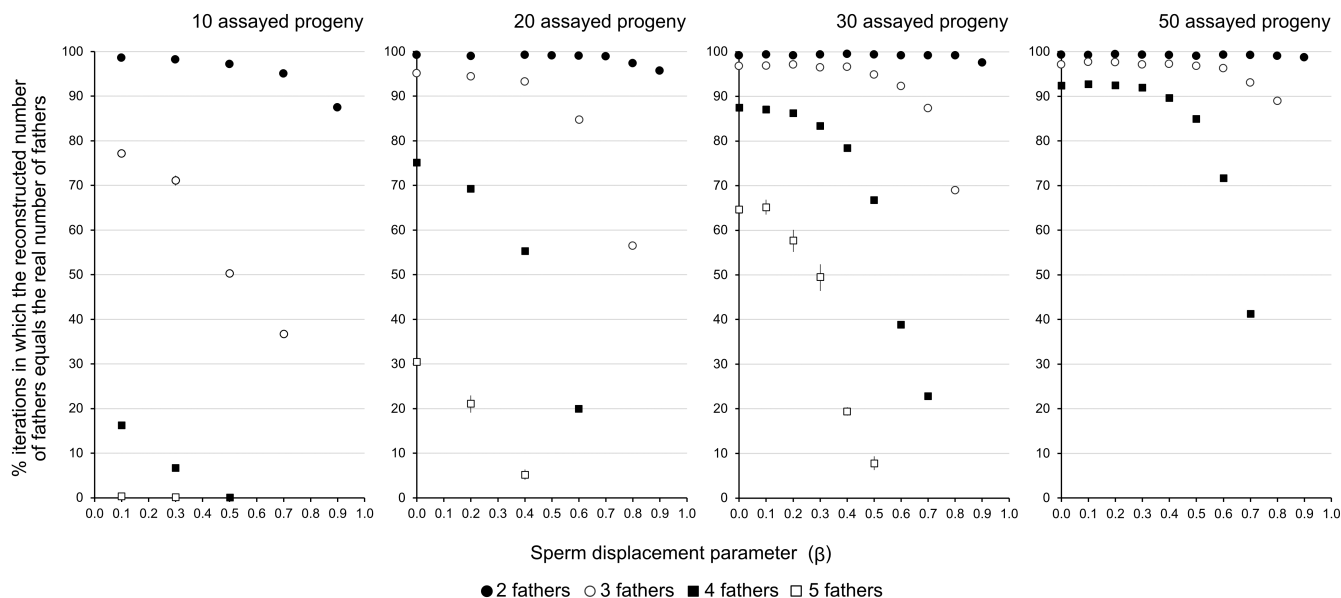


FIGURE 4 GERUDsim2.0 simulations of the ability of GERUD2.0 to correctly determine the number of fathers contributing to a family, considering different values of sperm displacement (β) and the number of progenies assayed. The mean number (\pm SE) of iterations in which the reconstructed number of fathers equalled the real number of fathers is indicated.

Scattered information is available on the physiological and molecular mechanisms that regulate mating behaviour and female remating (Krüger et al., 2019; Revadi et al., 2015; Xing et al., 2022; Young & Long, 2020). Female *D. suzukii* display an age-related mating activity with a significant increase in mating acceptance at 3 days of age (Revadi et al., 2015; Young & Long, 2020). Moreover, it has been suggested that *D. suzukii* females, in adverse conditions such as emergence from a bottleneck or the availability of low nutritional quality wild oviposition hosts, may be less choosy and may use a more indiscriminate mating strategy (Grassi et al., 2018; Krüger et al., 2019; Revadi et al., 2015; Young & Long, 2020). To determine the degree of polyandry, we considered a sample of the Costasavina population that was about to undergo a rapid demographic increase. It is reasonable to suppose that, at the time of our collections, the population was largely composed of young flies. Therefore, a physiological parameter, such as the average young age, may account for the high frequency of remating. Data on the role of female mating latency and on the mechanisms underpinning paternity allocation are scattered and discordant (Chen et al., 2022; Krüger et al., 2019). *Drosophila melanogaster* females can modulate their sperm use by adjusting their mating rate (Laturney et al., 2018). By mating with many males in quick succession, females may skew male–male sperm interactions, leading to a more equal share of paternity and thus greater progeny genetic diversity. Whether this behaviour is also adopted by *D. suzukii* females, with the consequent high remating rate and high shared paternity, is an open question. Indeed, *D. suzukii* differs with respect to *D. melanogaster* both in terms of ecology, physiology and reproductive potential (Little et al., 2020; Shearer et al., 2016; Tochen et al., 2014). It is thus conceivable that the mechanisms that control mating behaviour and paternity allocation differ in these two species. Knowledge of these mechanisms in *D. suzukii* is a priority,

not only for comparative purposes but also to interpret its reproductive potential in the context of population control programmes.

5 | CONCLUSIONS

A high degree of polyandry was detected during the steep demographic increase of the *D. suzukii* population after the winter bottleneck. Taking into account that polyandry reinforces the variability and the reproductive potential of a population (Holman & Kokko, 2013; Lewis et al., 2020; Olazcuaga et al., 2022), we might be led to speculate that this behaviour may be strategic to rapidly increase the reproductive potential and stability of the emerging summer populations. According to Vasudeva et al. (2021), polyandry may have adaptive significance if it is associated with an increase in male reproductive investment, ensuring elevated fertility and potential for adaptation. However, before considering this hypothesis, we admit that our data are related to only one point in the seasonal reproductive dynamics of this population (late spring/June). Only future analyses performed at different time points across the entire polyphenic reproductive cycle may help to determine the role of polyandry in this fly. In this context, it is noteworthy that the mean estimates for the number of sires in Costasavina were higher than those found in other *Drosophila* species such as *D. melanogaster*, *D. simulans* (Sturtevant, 1919), *D. buzzati* (Patterson & Wheeler, 1942), and *D. mojavensis* (Patterson, 1940), but are similar to those detected in a field population of *D. serrata* (Malloch, 1927), for which polyandry has been proposed to have an adaptive role (Frentiu & Chenoweth, 2008).

However, our results raise other important questions about the reproductive biology of *D. suzukii* and the use of control methods such as the Sterile Insect Technique (SIT). We have observed a sperm

displacement of 0.33, according to SCARE. However, the SCARE model assumes a last male advantage in siring the progeny, hence the term 'sperm displacement'. In the case of *D. sukuzii*, we have no information on whether it is the last or the previous males that have an advantage in siring the progeny. We do not know the molecular and physiological mechanisms underlying this high remating rate and sperm use. To answer these questions, controlled mating experiments are in progress. The resulting data will be of paramount importance for the application of the SIT as a control method. Knowing whether a multiply-mated female is able to differentially use the sperm from different males is essential to determining the size and frequency of sterile male releases in the target area.

AUTHOR CONTRIBUTIONS

ARM, RG, LMG, CC, ADC, CI and GG designed the study. SP, GF, AG, and RG conducted the field and laboratory work. SP, GF, LMG and DC analysed the data. ARM, SP and LMG wrote the manuscript. MVM helped with the revision of the manuscript. All authors read, made corrections and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Individual genotype data are available on DataDryad with the DOI <https://doi.org/10.5061/dryad.ttdz08m3b>.

BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases, as described above.

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SUPPORTING INFORMATION

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