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The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species

Joel Atallah, Lisa Teixeira, Raul Salazar, George Zaragoza and Artyom Kopp

Department of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA

Evolutionary innovation can allow a species access to a new ecological niche, potentially reducing competition with closely related species. While the vast majority of *Drosophila* flies feed on rotting fruit and other decaying matter, and are harmless to human activity, *Drosophila suzukii*, which has a morphologically modified ovipositor, is capable of colonizing live fruit that is still in the process of ripening, causing massive agricultural damage. Here, we conducted the first comparative analysis of this species and its close relatives, analysing both ovipositor structure and fruit susceptibility. We found that the ovipositor of the species most closely related to *D. suzukii*, *Drosophila subpulchrella*, has a similar number of enlarged, evolutionarily derived bristles, but a notably different overall shape. Like *D. suzukii*, *D. subpulchrella* flies are capable of puncturing the skin of raspberries and cherries, but we found no evidence that they could penetrate the thicker skin of two varieties of grapes. More distantly related species, one of which has previously been mistaken for *D. suzukii*, have blunt ovipositors with small bristles. While they did not penetrate fruit skin in any of the assays, they readily colonized fruit interiors where the skin was broken. Our results suggest that considering evolutionary context may be beneficial to the management of invasive species.

1. Introduction

One of the many advantages of *Drosophila* flies as model organisms for life science research has long been their benign relationship to our own species, allowing strains and transgenic stocks to be widely shared without the fear of jeopardizing either human endeavours or the natural environment [1]. The potential for conflict with humans was highlighted, however, following the 2008 identification of *Drosphila suzukii* in California [2]. In sharp contrast to the vast majority of *Drosophila* species, which feed on rotting fruit and other decaying vegetation, *D. suzukii*, a species that is native to east Asia and had not previously been identified on the US mainland, is capable of puncturing the skin of intact, ripening fruit to lay its eggs. Over the past 5 years, *D. suzukii* has spread widely across North America [3] and Europe [4], causing extensive agricultural damage [5]. Today, it ranks with the lionfish infestation of the western Atlantic [6] as one of the more severe ongoing biological invasions of the Western Hemisphere.

While there has been a proliferation of recent studies on the ecology and pest management of *D. suzukii* [7–11], this work has often been divorced from the broader context of *Drosophila* evolution. The ability of *D. suzukii* to lay its eggs in ripening fruit has been attributed to the unusual appearance of its ovipositor [3], but little research has been carried out on either the morphology or evolutionary origin of this structure. The evolutionary context, however, is critical from the perspective of both basic and applied science. From the vantage point of evolutionary theory, the derived ovipositor is an example of a putative key innovation [12], conferring an adaptive advantage by allowing *D. suzukii* to exploit a new ecological niche: young, undamaged fruit that is inaccessible to the larvae of other *Drosophila* species. From the applied science perspective, it is critical to know the extent to which other relatives of *D. suzukii* could behave as pests in a similar manner, in the hope of

preventing their spread before they are established. Indeed, popular guides have referred to *D. suzukii* as 'spotted wing *Drosophila*' [3,5,13] but this description applies to a number of flies in this species group [14,15] and it is not clear how many of them are potentially harmful to agriculture.

We carried out a comparative study of fruit susceptibility to *D. suzukii* and three of its closest relatives (*Drosophila subpulchrella*, *Drosophila biarmipes* and *Drosophila mimetica*) [16,17], and combined this work with a morphological analysis of their ovipositors. In our experiments, only *D. suzukii* and *D. subpulchrella* (figure 1), the two species with ovipositors that carry enlarged bristles, punctured the intact skin of raspberries and cherries. However, while the number and morphology of enlarged bristles does not differ between these species, only *D. suzukii* punctured the tough skin of grapes. The shape of the *D. suzukii* ovipositor differs from the three other species, suggesting that changes in ovipositor shape evolved after the evolution of enlarged bristles. Our results show that *D. subpulchrella* could be a significant threat to the raspberry and cherry industries, while suggesting that other closely related species, including one with a spotted wing (*D. biarmipes*), are unlikely to be harmful.

2. Material and methods

(a) Fruit susceptibility experiments

The susceptibility of four varieties of fruit to flies of four species (*D. mimetica*, *D. biarmipes*, *D. subpulchrella* and *D. suzukii*) was assayed. All flies were cultured on standard laboratory media. Bottles of flies containing pupae ready to eclose were emptied of all adults. Five to 7 days later, any adults that had emerged from the pupae were transferred to separate bottles and were aged for another 6–7 days. This process ensured that all flies were between 6 and 14 days old prior to the start of the experiment.

For each experiment, three female flies of each of the four species were placed in separate plastic bottles with foam plugs, with each bottle containing one raspberry, cherry, red grape or Thompson grape. (Thompson grapes, also referred to as sultana grapes, are a green variety that is typically tarter than the red grapes. Both varieties are grown in California.) Only fresh fruits were used. After 24 h of exposure to the fruit, flies were removed and each fruit was analysed under dissecting microscopes by two raters. In some cases, particularly for *D. mimetica*, we observed that flies had died during the 24 h period. Eggs (or hatched larvae) in the exposed region of the fruit (almost always the pit of the fruit, but including in a few cases other areas where the skin had broken naturally prior to the start of the experiment) were counted separately from eggs found in the intact region of the skin, inserted through punctures generated by the fly (figures 2 and 3). The identification of eggs was facilitated by the presence of protruding filaments (extensions of the chorion). Punctures without eggs (displaying no filaments) were counted in a separate tally. In cases where the raters failed to reach a consensus, the mean value of the two counts was used. Ten to 11 simultaneous replicates of each experiment were carried out.

Only *D. suzukii* flies laid eggs in the intact region of Thompson grapes (figure 3*b*), and these were very rare. Therefore, in order to have enough punctures with eggs to make meaningful comparisons with the punctures without eggs (figure 3*d*), we carried out a separate experiment where we placed 6–10
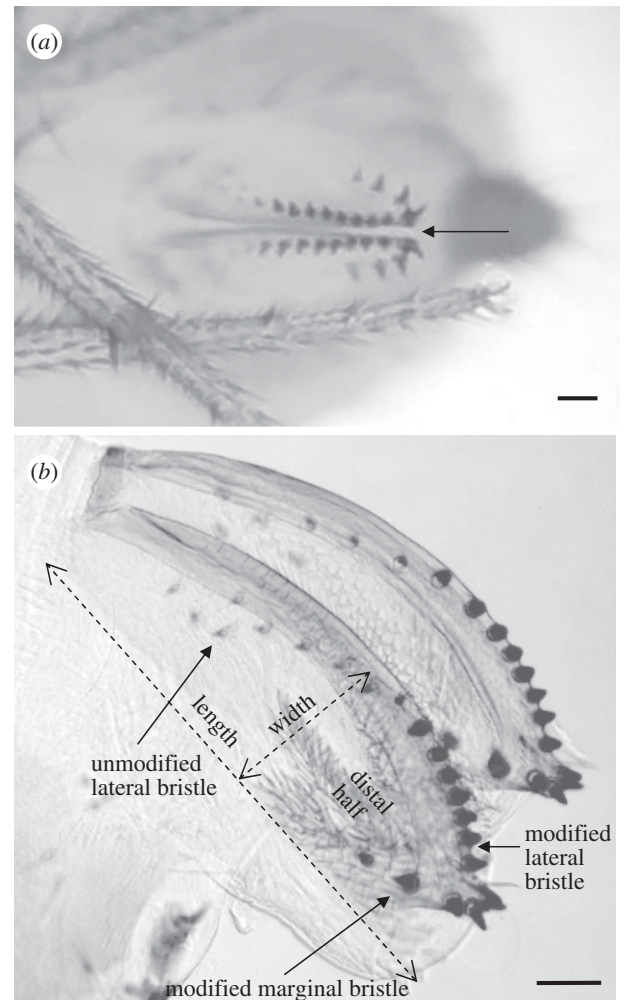

Figure 1. A serrated ovipositor. (*a*) Ventral view of the posterior region of *D. subpulchrella*, showing the ovipositor consisting of a bilateral pair of sclerotized plates (arrow). (*b*) A lateral view of a dissected *D. subpulchrella* ovipositor, with both plates visible. Note the enlarged, strongly pigmented modified bristles in both the lateral and marginal regions of the distal portion of the ovipositor. These contrast sharply with the unmodified proximal bristles, which are similar to the ancestral ovipositor bristle phenotype. The figure also shows the length and width of an ovipositor plate, and the position of the 'distal half', as defined in this study for the purposes of morphological analysis (see Material and methods). Scale bars, 50 μm.

D. suzukii female flies per bottle. We measured the area of a total of 18 randomly selected punctures with egg filaments and compared the results to 14 punctures without filaments from the same experiment.

(b) Morphological analysis of the ovipositors

Two strains of each of the four species were used for the morphological analysis (electronic supplementary material, table S1). Ten ovipositor plates from each strain, each from a separate fly, were analysed, and the total number of bristles on each plate was determined (figure 4*a*). Some of the ovipositor bristles on *D. subpulchrella* and *D. suzukii* are modified, being enlarged and heavily pigmented. These modified bristles were counted and the location of each bristle (lateral or marginal; figure 1*b*) was recorded (figure 4*b*).

Outlines of the ovipositor plates were generated manually from photographs. In a manner analogous to a study of the posterior lobe of the male genitalia [18,19], a horizontal
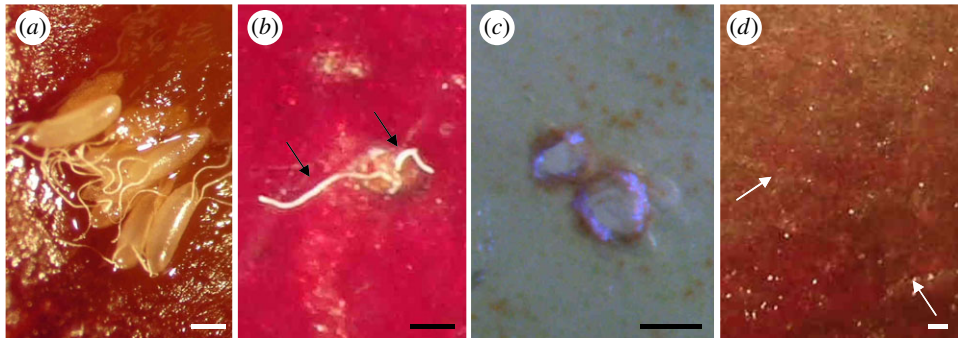
Figure 2. Oviposition and attempted oviposition on fruit. The fruit and species are in parentheses. (*a*) Eggs laid at the damaged (exposed) region of a fruit (cherry; *D. biarmipes*). (*b*) A fruit punctures in previously intact skin with telltale filaments (arrows), evidence of an egg embedded in the fruit body (cherry; *D. suzukii*). (*c*) Fruit punctures without egg filaments (Thompson grape; *D. suzukii*). (*d*) Scratches on a fruit (arrows), possible evidence of a failed attempt to create a puncture (red grape; *D. subpulchrella*). Scale bars, 200 μm. (Online version in colour.)
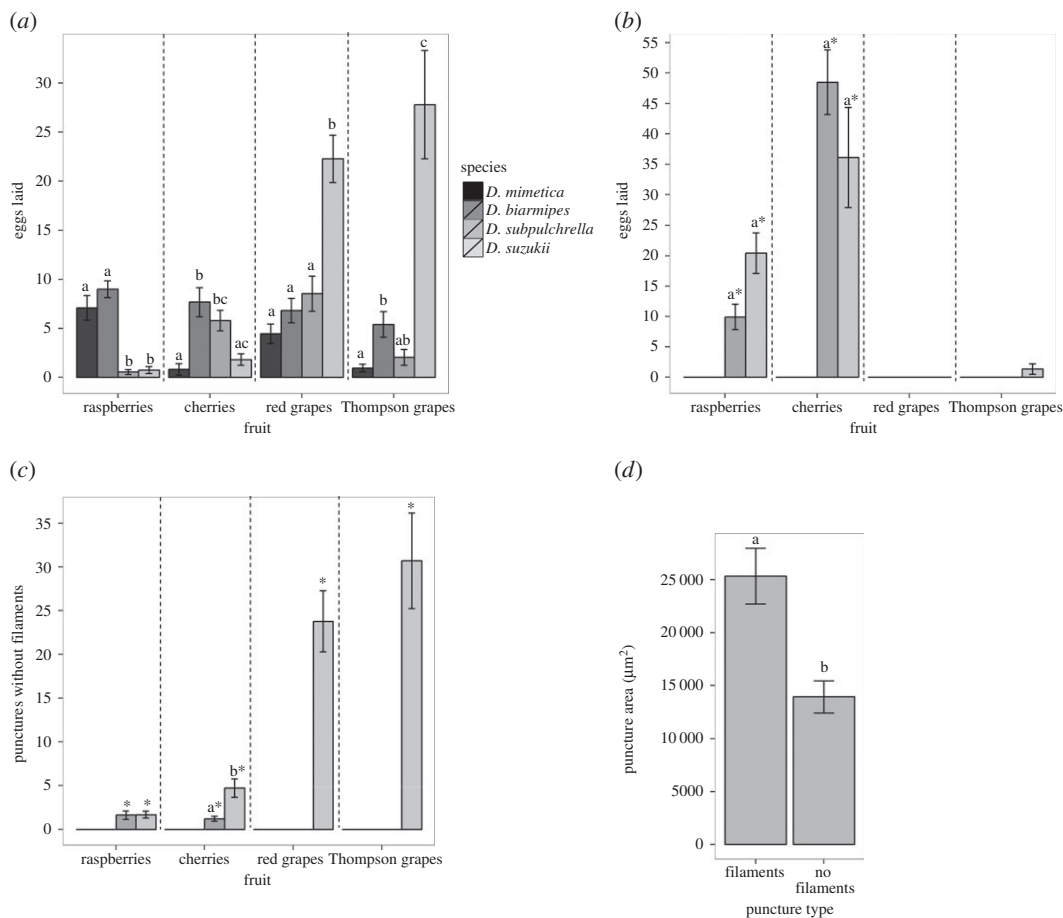

Figure 3. The effects of exposing four varieties of fruit to four species of *Drosophila*. Means are graphed with the error bars showing the standard error of the mean. Different letters indicate significant differences at the 0.05 level using pairwise Mann–Whitney *U*-tests with the Bonferroni correction for multiple comparisons. An asterisk indicates a significant difference from zero. Bar charts in panels (*a*–*c*), described by the key to the right of panel (*a*), show the number of punctures or eggs laid per fruit. (*a*) Eggs laid in exposed regions of the fruit. (*b*) Eggs laid in the intact region of the fruit body. (*c*) Punctures without filaments. (*d*) A comparison of the relative area of punctures with and without filaments in Thompson grapes exposed to *D. suzukii*, the only species that was successful at puncturing grapes. The data for this panel were obtained in a separate experiment in which *D. suzukii* flies were exposed to the grapes at high density (see Material and methods for details).

line was drawn at the base of the ovipositor plate where the pigmentation fades and the structure merges with the abdomen (figure 1*b*). The area and length to width ratio (figures 1 and 4*c*,*d*) of each plate outline were calculated using the program IMAGEJ. The same program was also used to calculate puncture area (figure 3*d*) and wing area (electronic supplementary material, figure S1).

Although the flies we studied are all closely related, the ovipositors do not contain easily identifiable landmarks that are

invariant across species, making it difficult to employ standard landmark-based morphometric techniques. We decided, therefore, to use elliptical Fourier analysis (EFA) [20], which does not require the identification of landmarks. The technique uses a series of contours, described by Fourier harmonics, to approximate a shape. Each harmonic is specified by four Fourier coefficients. Following the example of previous studies [18,19,21], we decided to use 25 harmonics. We conducted the EFA (figure 5) on the distal half of the ovipositor
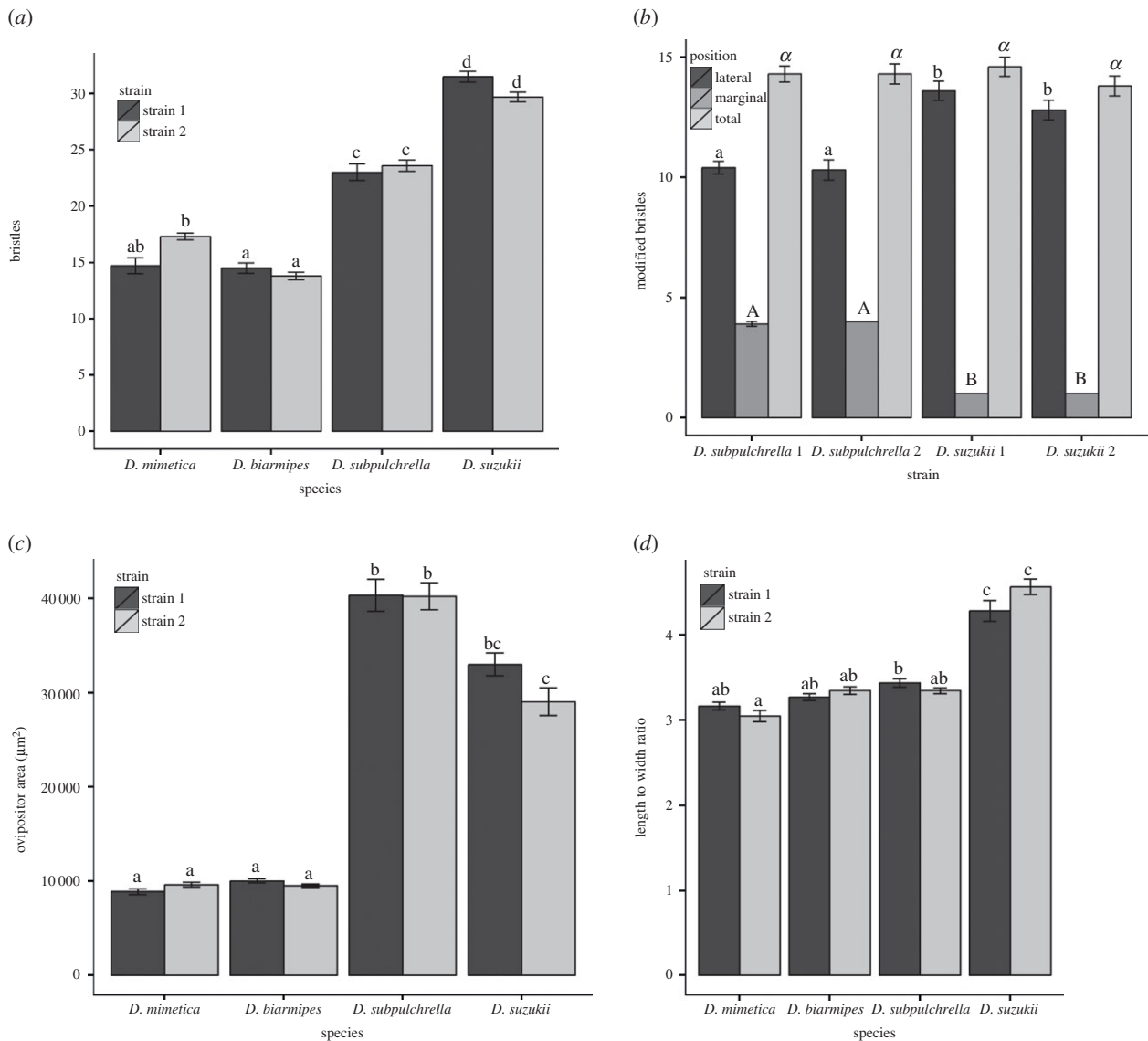
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Figure 4. A comparison of ovipositor morphology in the four species. Two strains from each species (electronic supplementary material, table S1) were used for this comparison. Means are graphed and the error bars show the standard error of the mean. Different letters indicate significant differences at the 0.05 level using pairwise Mann–Whitney U-tests with the Bonferroni technique to correct for multiple comparisons. (*a*) The total number of bristles per ovipositor plate. (*b*) The number of modified bristles, in *D. subpulchrella* and *D. suzukii*, on three regions of the plate (see figure 1). Lower-case Latin letters are used to indicate statistically significant differences for the lateral region, capital Latin letters for the marginal region and Greek letters for the total number of modified bristles. (*c*) Ovipositor plate area. (*d*) Ovipositor length to width ratio.

plates (figure 1), because this is the portion that comes into contact with the fruit.

As it is difficult to compare outlines on a large number of Fourier coefficients (100 if 25 harmonics are used), principal component analysis (PCA) is typically used to reduce the data to an orthogonal set of variables ordered according to the proportion of variation explained [22]. As the interpretation of the principal components (PCs), however, is not immediately clear, we reconstructed the outlines explained by each PC using the inverse Fourier transform (see figure 5*b*).

The software package SHAPE [23] was used for the EFA and PCA.

(c) Statistical analyses

All statistical analyses were carried out using the programming language R. The fruit experiments were designed specifically to compare the susceptibility of the exposed and intact region of each type of fruit across the four species. We therefore

tested the following model: number of eggs = f(species), considering each fruit and skin condition (exposed versus intact) separately. The Kruskal–Wallis analysis of variance, a nonparametric equivalent of ANOVA, was used to compare groups across species. When significant, this test was followed by pairwise Mann–Whitney U-tests, and the p-values were corrected for multiple comparisons using the Bonferroni technique. In this and subsequent analyses, the Bonferroni adjustment multiplies the p-values we obtained by the number of comparisons performed (e.g. six comparisons when comparing across four species).

For the morphological comparisons, we tested the following models: number of bristles (total or modified) = f(strain) (figure 4*a*,*b*) and ovipositor area (or length/width ratio) = f(strain). The Kruskal–Wallis analysis of variance was used to compare groups across strains. When significant, this test was followed by pairwise Mann–Whitney U-tests, and the p-values were corrected for multiple comparisons using the Bonferroni technique.
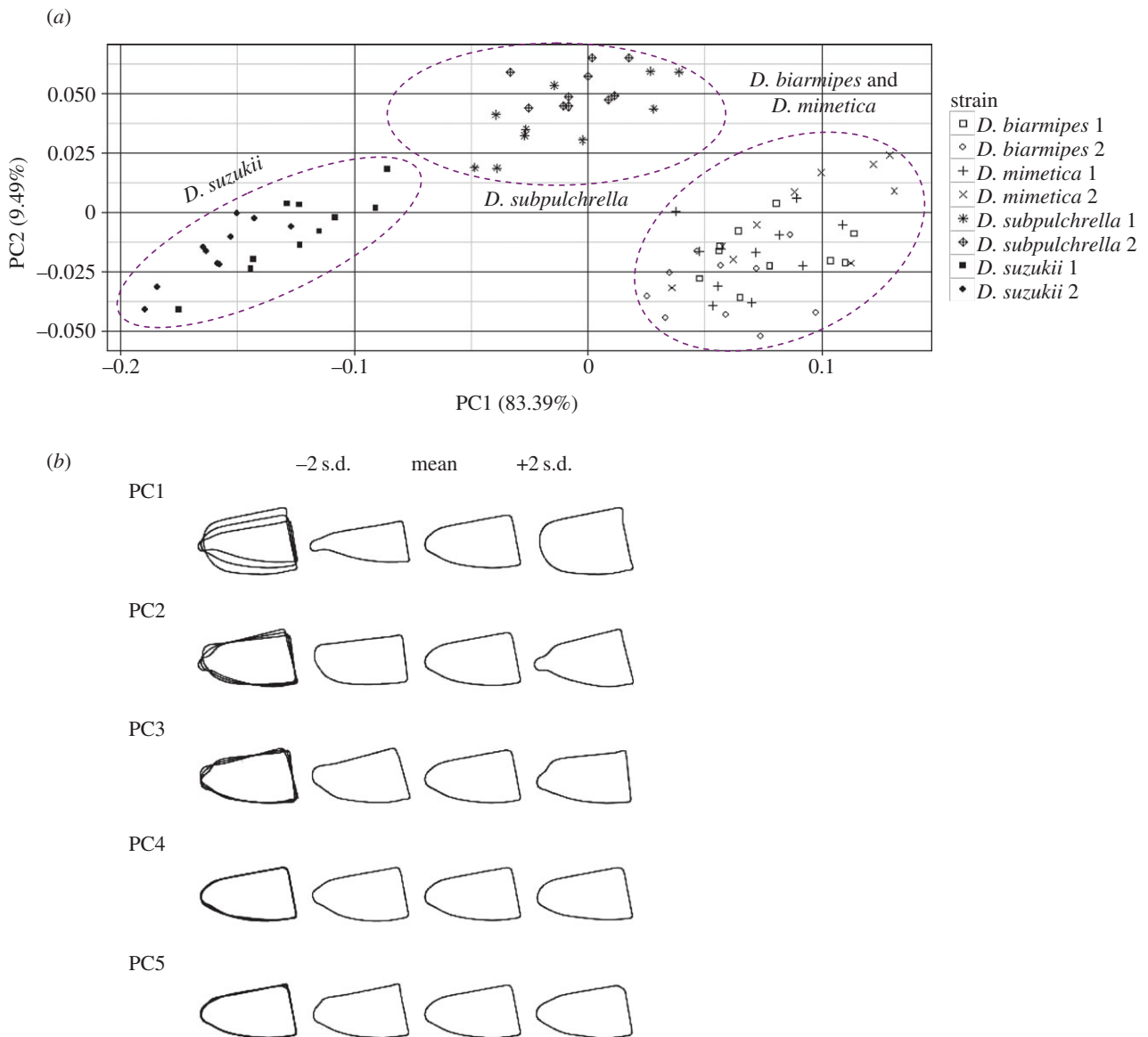
Figure 5. EFA of the distal half of ovipositor plate outlines. (a) First (x-axis) and second (y-axis) PC scores of the EFA. (b) Reconstructions of outlines using the inverse Fourier transform, based on the mean value, and the mean ± 2 s.d., of each of the first five PCs. The left column is a superposition of the three reconstructed outlines. (Online version in colour.)

3. Results

(a) All species will lay their eggs in damaged regions of all fruits tested

We sought to determine the susceptibility of four types of fruit to each of the four species of flies. They included a fruit with relatively soft skin (raspberries) and fruits with much tougher skin (two varieties of grapes). Our aim was to discover whether females of each species were capable of penetrating the intact skin of ripening fruit. We were aware, however, that the failure of females of a given species to lay eggs in a fruit could be a sign of either their inability to puncture the skin or a general aversion to fruit of that variety. To distinguish between these possibilities, we counted separately the number of eggs in the exposed region of the fruit, which included the pit and any regions where the skin had naturally broken, and the undamaged fruit body. The absence of eggs in both the easily accessible exposed region and the unexposed area would indicate an aversion to the fruit, while the presence of eggs in the exposed area, and

their absence wherever the skin was intact, would be consistent with the hypothesis that the skin was acting as a barrier.

We found that all four species of flies laid eggs in the exposed portion of the four fruit varieties (figure 3a), indicating that none of the species had a strong aversion to using these fruit as larval food sources. The eggs that were laid in this region were found in a number of positions. The egg was sometimes embedded in the fruit with the respiratory filaments pointing outwards, but it could also be found in the opposite orientation or lying sideways (figure 2a). In the raspberry assay, a significantly larger number of eggs were laid in the exposed region by D. mimetica and D. biarmipes females when compared with D. suzukii and D. subpulchrella (figure 3a). This may be a consequence of the fact that the latter two species appear to have had no difficulty puncturing intact raspberry skin (see §3b), and therefore did not gravitate to the far smaller exposed area in the pit. These results contrast with the data from both varieties of grapes, where all four species laid eggs in the exposed portion, but D. suzukii laid over three times as many as the other three species. In the case of cherries, both
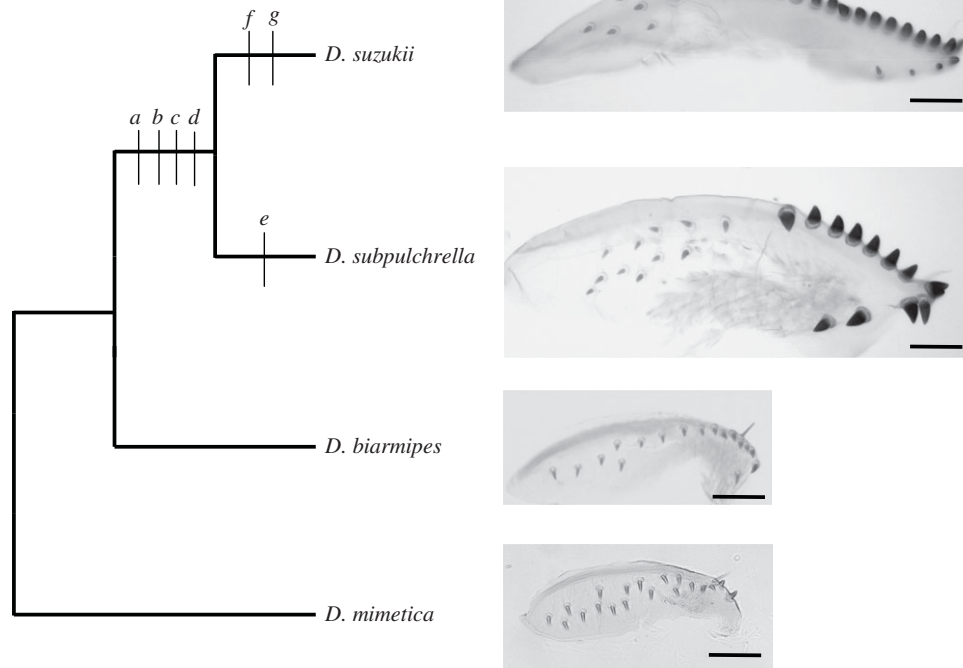
Figure 6. A model of ovipositor evolution. The phylogeny is based on previous work [16,17], and only the topology is shown. Lettering is used to indicate the timing of specific evolutionary changes. (*a*) Increase in ovipositor area. (*b*) Evolution of modified (enlarged, pigmented) ovipositor bristles. (*c*) Evolution of a sharper ovipositor tip. (*d*) Evolution of the ability to puncture the skin of raspberries and cherries. (*e*) Evolution of a distal bulb. (*f*) Evolution of a streamlined ovipositor (increased length to width ratio). (*g*) Evolution of the ability to puncture the skin of grapes. Scale bars, 50 μm.

D. biarmipes and *D. subpulchrella* laid significantly more eggs in the exposed area than *D. mimetica* (figure 3*a*).

(b) Egg laying in the intact skin of raspberries and cherries was limited to *Drosophila suzukii* and *Drosophila subpulchrella*

In the raspberry and cherry assays, only *D. suzukii* and *D. subpulchrella* laid eggs in areas of the fruit where the skin was intact (figure 3*b*). There was no significant difference between these two species in the number of eggs laid in either fruit. We also found cases of punctures that lacked egg filaments (figures 2*c* and 3*c*), a possible indication that an egg-laying attempt was unsuccessful. These were relatively rare in both the raspberry and cherry assays (figure 3*c*), suggesting that neither species had much difficulty laying their eggs in these fruits.

The finding that *D. subpulchrella* flies are capable of puncturing the skin of cherries and raspberries raises the question of whether this species, like *D. suzukii*, could be a threat to soft-fruit industries. We therefore endeavoured to determine whether *D. subpulchrella* larvae could survive and develop in the pulp of these fruits. For the cherry assays, we observed an average of 19.9 living larvae in each fruit 5 days after the assays were set up (an average survivability of 36.6%). For the raspberry assays, we observed the bottles 14 days after they were set up and found that an average of seven adult flies had emerged from pupae. This indicates that larvae can survive in both of these fruits and, at least in raspberries, can develop to adulthood.

No punctures of any form were found in the intact regions of either raspberries or cherries exposed to *D. mimetica* and *D. biarmipes*. In conjunction with the results of §3*a*, the findings are consistent with the hypothesis that these species rarely or never puncture intact skin even in soft-skinned fruits and that the propensity to do so evolved in the ancestor of *D. suzukii* and *D. subpulchrella* (figure 6).

(c) Relatively few eggs were laid in grape skin

The results of the two grape assays (figure 3*a*–*c*) differed strongly from the other fruits. Only *D. suzukii* laid eggs through the intact skin of any grapes, but these were relatively rare in the Thompson grapes, and entirely absent from the red grapes. However, grapes exposed to *D. suzukii* showed numerous punctures without the telltale sign of egg filaments (figure 3*c*). The egg-free punctures were often found in clusters. We determined, in a separate experiment (see Material and methods), that punctures without protruding egg filaments were considerably smaller than those with filaments (figure 3*d*). These findings are consistent with the hypothesis that *D. suzukii* females tried to lay their eggs in the grape bodies and often succeeded in making small holes in the skin, but in most cases failed to insert an egg. However, from a pest-management perspective, it is important to note that punctures without filaments can be sites of secondary infections.

By contrast, the intact body regions of grapes exposed to flies of other species never showed punctures. However, in a few cases, we found evidence of 'slashes' in the bodies of grapes in bottles with either *D. subpulchrella* or *D. suzukii* (figure 2*d*, arrow). The slashes, which were not found in control fruits that were not exposed to these flies, may represent unsuccessful attempts to puncture the skin.

Our results demonstrate that while all species have an affinity for grapes and will lay their eggs in exposed regions of the fruit, they rarely penetrate the intact skin of the two varieties we tested. The evidence of egg-laying attempts, especially in *D. suzukii*, suggests that a property of the skin (e.g. its

thickness or texture) may make the insertion of eggs difficult. The *D. suzukii* findings are consistent with the results of previous research [24].

(d) Evolution of ovipositor morphology

The fruit susceptibility experiments suggest that the ability to puncture fruit skin first evolved in, or at least was strongly enhanced in, the ancestor of *D. suzukii* and *D. subpulchrella* (figure 6). We tested whether this capacity was accompanied by concomitant phenotypic changes in the structure of the ovipositor.

We separated, mounted and imaged ovipositor plates from two strains of each of the four species and compared them on a number of traits. One categorical distinction between ovipositors in the *D. suzukii* and *D. subpulchrella* clade, on the one hand, and *D. mimetica* and *D. biarmipes*, on the other, is the presence of thick, pigmented bristles on the former, explaining why this type of ovipositor has been referred to as 'serrated' (figure 6). These modified bristles are found close to the distal tip of the ovipositor (figure 1), which comes into contact with fruit, while the more proximal bristles are unmodified and resemble the homologous bristles present on the ovipositors of other species, referred to in the literature as 'thorn bristles' [25]. The evolution of modified bristles in this clade was accompanied by a significant increase in the overall number of thorn bristles (figure 4a). Interestingly, the three to four marginal bristles of the *D. subpulchrella* ovipositor tip are generally of the thick, pigmented variety, whereas those in the equivalent region of the *D. suzukii* plate are almost always unmodified (figures 1, 4b and 6). However, on the lateral side of the ovipositor, *D. suzukii* has more modified bristles than *D. subpulchrella*, and the total number of modified bristles does not differ significantly between the two species (figure 4b).

The evolution of the serrated ovipositor was accompanied by a considerable increase in the ovipositor size (figure 4c), with the two species with the serrated variety having ovipositor plates with approximately three to four times the area of the two other species' plates. By contrast, comparing the same species on wing area, often used as a proxy for body size [26], shows an increase of only 1.6-fold to twofold in *D. suzukii* and *D. subpulchrella* (electronic supplementary material, figure S1; some interstrain variation in wing area is apparent). Therefore, while there has been an overall increase in body size in *D. suzukii* and *D. subpulchrella*, there has been a disproportionate increase in ovipositor area.

In order to have a quantitative method of distinguishing 'sharp' ovipositors from 'blunt' ones, we measured the length to width ratio (figures 1 and 4d). While this ratio does not vary significantly among *D. mimetica*, *D. biarmipes* and *D. subpulchrella* (figure 4d), it increased markedly in *D. suzukii*, giving the egg-laying organ a pointed, streamlined shape (figure 6).

We used EFA to compare the ovipositor shape among the four species, focusing on the distal half of the structure (figure 1b), because this is the portion of the egg-laying organ that comes into contact with the fruit during an attempted puncture. EFA produced a series of four Fourier coefficients for each of the 25 harmonics used in the analysis. Using the PCA, we calculated PCs for each of the ovipositors (electronic supplementary material, table S2). The vast majority (93%) of the variation was captured by the first two PCs, which are plotted in figure 5a. Three groupings

are apparent in this figure, each indicated with an ellipse: the *D. mimetica* and *D. biarmipes* strains; the *D. subpulchrella* strains; and the *D. suzukii* strains. No significant differences are seen between the two strains of each species (electronic supplementary material, tables S3–S5).

To help to visualize what the individual PCs represent, we used the inverse Fourier transform to reconstruct ovipositor outlines on the basis of each PC (figure 5b). The outlines suggest that the first PC (top line in figure 5b) represents the difference between a sharp and blunt ovipositor. Indeed, we found that there is a strong negative Pearson product correlation (0.89) between the first PC and length to width ratio. Therefore, the horizontal axis of figure 5a shows that *D. biarmipes* and *D. mimetica*, the two species that did not puncture the intact skin of any of the fruits tested, both have relatively blunt ovipositors and do not differ significantly on this variable (electronic supplementary material, table S3). *Drosophila subpulchrella* has a sharper distal ovipositor, whereas the ovipositor of *D. suzukii* is the sharpest of all.

A positive value for the second PC (figure 5b) is indicative of an ovipositor with a bulb at the tip, as is seen in *D. subpulchrella* (figures 1 and 6). Not surprisingly, there is a strong, significant difference between the *D. subpulchrella* strains and those of the other three species on this PC (figure 5a, y-axis; electronic supplementary material, table S4), while the latter do not vary significantly among each other on this variable (with the exception of the second *D. biarmipes* strain and the second *D. mimetica* strain). The third PC (figure 5b; electronic supplementary material, table S5), which only explains about 4% of the variation (electronic supplementary material, table S2), appears reflective of the asymmetry in the direction in which the tip is pointed. The remaining PCs each accounted for less than 1% of the variation, and the use of an omnibus Kruskal–Wallis test found no evidence of significant differences between strains.

We present a model for the evolution of a modified ovipositor in figure 6 (see also the expanded phylogeny in the electronic supplementary material, figure S2). The appearance of enlarged bristles was accompanied by a change in the shape of the ovipositor tip, which became significantly sharper. Following this event, in one species, *D. subpulchrella*, a distal bulb appeared, while in the second species, *D. suzukii*, the ovipositor became even sharper and increasingly streamlined. The latter is associated with the ability of *D. suzukii* to puncture the skin of the grape varieties we assayed, which was not shared by the other species in this study.

4. Discussion

While there were numerous Japanese studies of *D. suzukii* infestations during the first half of the twentieth century (reviewed in [2]), these reports were not widely disseminated among Western researchers. When this species was first collected in California in 2008, it was not readily identified and was initially mistaken for *D. biarmipes* [2]. At the time, the only known drosophilid pest in the Western Hemisphere was the distantly related *Zaprionus indianus*, which had first been reported in North America in 2006 [27], 7 years after its identification in Brazil [28]. Although *Z. indianus* is a formidable threat to agriculture (particularly figs, where the ostiole allows the larvae easy access to the pulp), it can only colonize exposed fruit [27,29]. Other distantly related species, however, such as

the leaf-mining fly *Scaptomyza flava*, possess serrated ovipositors [30]. In this species, the ovipositor is used to puncture a leaf, and the female then feeds on its contents.

The work reported here has focused on addressing two issues: which species will puncture intact fruit skin and the morphological evolution of the ovipositor. Future research, carried out in a comparative context, may lead to more insights on both the agricultural and evolutionary implications of our findings. A thorough analysis of any interspecific differences in both adult and larval survivability, for example, and whether larval development in the fruit pulp varies depending on whether the egg was laid in an exposed area or through a skin puncture, will aid in the assessment of the agricultural threat, including the risk of secondary invasions (reviewed in [31]). Behavioural studies of egg-laying attempts will help to determine the extent to which the fly's propensity to oviposit (or attempt to oviposit) in intact skin has coevolved with ovipositor morphology. In our own preliminary observations of the flies' behaviour when trying to oviposit, we have observed that flies stab repeatedly at fruit skin with their ovipositors, apparently attempting to lay an egg, but sometimes only succeeding (if at all) after numerous failed attempts. Detailed analyses of both successes and failures may help to resolve the issue of whether the failure to puncture the skin is owing to morphological limitations of the ovipositor or an aversion to the skin.

One model of adaptive evolution, dating back to George Gaylord Simpson, posits that a key innovation can provide a novel ecological opportunity leading to diversification [32] (see also [12]). However, we have knowledge of only one other closely related species, *Drosphila pulchrella* [15], with putatively enlarged thorn bristles (*D. pulchrella* and *D. subpulchrella* were not distinguished prior to 2006, and no verified cultures of *D. pulchrella* are currently available for analysis). Therefore, there is little evidence in this case of an 'adaptive radiation' of species following the innovation, as the classic model would posit [33].

It would appear to be surprising for an innovation that confers such a strong adaptive advantage to be rare. The pattern in Drosophilidae makes for an instructive contrast with another lineage, the fly superfamily Tephritoidea, where the evolution of an ovipositor optimized for piercing tough plant surfaces spawned a radiation of thousands of species [34]. Ovipositor modification has also played a key role in the natural history of other insect taxa, including the aculeate Hymeonoptera, where the organ has evolved into a sting [35]. The species we have analysed here, however, are much more closely related to *Drosophila melanogaster* than any other cases where the ecological importance of ovipositor evolution has been demonstrated, facilitating the transfer of the vast array of resources developed for the model fruitfly. Analysis of the developmental genetics of ovipositor ontogeny, and interspecific comparisons of this genetic circuitry, along with new genomic resources [36], may shed light on how the structure can be evolutionarily co-opted. It is possible that this system could become a promising model for evolutionary developmental biology.

We have reported here an assessment of the potential of a *Drosophila* species other than *D. suzukii* to puncture fruit skin. We have found that while the capabilities of *D. subpulchrella* differ from those of *D. suzukii*, it nonetheless has the ability—in apparent contrast to more distant relatives of *D. suzukii*—to penetrate the exterior of ripening raspberries and cherries. As little is known of the ecology of this species, it is difficult to know whether it has invasive potential. Vast resources have been devoted to the study of *D. suzukii* in recent years, with no comparable resources being given to *D. subpulchrella* research. Our results suggest that applying an evolutionary framework in pest management is both prudent and practical.

References

1. Ashburner M, Golic K, Hawley RS. 2004 Drosophila: a laboratory handbook, 2nd edn. New York, NY: Cold Spring Harbor Laboratory Press.

2. Hauser M. 2011 A historic account of the invasion of Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag. Sci. 67, 1352–1357. (doi:10.1002/ps.2265)

3. Lee JC, Bruck DJ, Dreves AJ, Ioriatti C, Vogt H, Baufeld P. 2011 In focus: spotted wing drosophila, Drosophila suzukii, across perspectives. Pest Manag. Sci. 67, 1349–1351. (doi:10.1002/ps.2271)

4. Cini A, Ioriatti C, Anfora G. 2012 A review of the invasion of Drosophila suzukii in Europe and a draft research agenda for integrated pest management. Bull. Insectol. 65, 149–160.

5. Landolt PJ, Adams T, Davis TS, Rogg H. 2012 Spotted wing drosophila, Drosophila suzukii (Diptera: Drosophilidae), trapped with combinations of wines and vinegars. Fla. Entomol. 95, 326–332. (doi:10.1653/024.095.0213)

6. Green SJ, Akins JL, Maljković A, Côté IM. 2012 Invasive lionfish drive Atlantic coral reef fish declines. PLoS ONE 7, e32596. (doi:10.1371/journal.pone.0032596)

7. Mitsui H, Beppu K, Kimura MT. 2010 Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol. Sci. 13, 60–67. (doi:10.1111/j.1479-8298.2010.00372.x)

8. Haviland DR, Beers EH. 2012 Chemical control programs for Drosophila suzukii that comply with international limitations on pesticide residues for exported sweet cherries. J. Integr. Pest Manag. 3, F1–F6. (doi:10.1603/IPM11034)

9. Chabert S, Allemand R, Poyet M, Eslin P, Gibert P. 2012 Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, Drosophila suzukii. Biol. Control 63, 40–47. (doi:10.1016/j.biocontrol.2012.05.005)

10. Maier CT. 2012 First detection and widespread distribution of the spotted wing drosophila, Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), in Connecticut in 2011. Proc. Entomol. Soc. Wash. 114, 329–337. (doi:10.4289/0013-8797.114.3.329)

11. Burrack HJ, Fernandez GE, Spivey T, Kraus DA. 2013 Variation in selection and utilization of host crops in the field and laboratory by Drosophila suzukii Matsumara (Diptera: Drosophilidae), an invasive frugivore. Pest Manag. Sci. 69, 1173–1180. (doi:10.1002/ps.3489)

12. Losos JB. 2010 Adaptive radiation, ecological opportunity, and evolutionary determinism. Am. Nat. 175, 623–639. (doi:10.1086/652433)

13. Goodhue RE, Bolda M, Farnsworth D, Williams JC, Zalom FG. 2011 Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manag. Sci. 67, 1396–1402. (doi:10.1002/ps.2259)

14. Bock IR, Wheeler MR. 1972 The Drosophila melanogaster species group. Univ. Tex. Publ. 7213, 1–102.

15. Takamori H, Watabe H, Fuyama Y, Zhang Y, Aotsuka T. 2006 *Drosophila subpulchrella*, a new species of the *Drosophila suzukii* species subgroup from Japan and China (Diptera: Drosophilidae). *Entomol. Sci.* **9**, 121–128. (doi:10.1111/j.1479-8298.2006.00159.x)

16. Barmina O, Kopp A. 2007 Sex-specific expression of a HOX gene associated with rapid morphological evolution. *Dev. Biol.* **311**, 277–286. (doi:10.1016/j.ydbio.2007.07.030)

17. Prud'Homme B, Gompel N, Rokas A, Kassner VA, Williams TM, Yeh S-D, True JR, Carroll SB. 2006 Repeated morphological evolution through cis-regulatory changes in a pleiotropic gene. *Nature* **440**, 1050–1053. (doi:10.1038/nature04597)

18. Liu J, Mercer JM, Stam LF, Gibson GC, Zeng Z-B, Laurie CC. 1996 Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritiana*. *Genetics* **142**, 1129–1145.

19. McNeil CL, Bain CL, Macdonald SJ. 2011 Multiple quantitative trait loci influence the shape of a male-specific genital structure in *Drosophila melanogaster*. *G3 Genes Genomes Genet.* **1**, 343–351. (doi:10.1534/g3.111.000661)

20. Kuhl FP, Giardina CR. 1982 Elliptic Fourier features of a closed contour. *Comput. Graph. Image Process.* **18**, 236–258. (doi:10.1016/0146-664X(82)90034-X)

21. Macdonald SJ, Goldstein DB. 1999 A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. *Genetics* **153**, 1683–1699.

22. Furuta N, Ninomiya S, Takahashi S, Ohmori H, Ukai Y. 1995 Quantitative evaluation of soybean (*Glycine max* L. Merr.) leaflet shape by principal component scores based on elliptic Fourier descriptor. *Breed Sci.* **45**, 315–320.

23. Iwata H, Ukai Y. 2002 SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered.* **93**, 384–385. (doi:10.1093/jhered/93.5.384)

24. Lee JC, Bruck DJ, Curry H, Edwards D, Haviland DR, Van Steenwyk RA, Yorgey BM. 2011 The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Manag. Sci.* **67**, 1358–1367. (doi:10.1002/ps.2225)

25. Chen EH, Baker BS. 1997 Compartmental organization of the *Drosophila* genital imaginal discs. *Development* **124**, 205–218.

26. Partridge L, Barrie B, Fowler K, French V. 1994 Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**, 1269–1276. (doi:10.2307/2410384)

27. Van der Linde K, Steck GJ, Hibbard K, Birdsley JS, Alonso LM, Houle D. 2006 First records of *Zaprionus indianus* (Diptera: Drosophilidae), a pest species on commercial fruits from Panama and the United States of America. *Fla. Entomol.* **89**, 402–404. (doi:10.1653/0015-4040(2006)89[402:FROZID]2.0.CO;2)

28. Vilela CR. 1999 Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region? *Drosoph. Inf. Serv.* **82**, 37–39.

29. Raga A, de Souza Filho MF. 2003 Captura de *Zaprionus indianus* (Gupta) (Dip.: Drosophilidae) em frascos de plasticos com iscas alimentares na cultura do figo. *Rev. Agric. Piracicaba* **78**, 323–329.

30. Whiteman NK *et al*. 2011 Mining the plant–herbivore interface with a leafmining *Drosophila* of *Arabidopsis*. *Mol. Ecol.* **20**, 995–1014. (doi:10.1111/j.1365-294X.2010.04901.x)

31. Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, Walton VM, O'Neal SD, Zalom FG. 2011 *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *J. Integr. Pest Manag.* **2**, G1–G7. (doi:10.1603/IPM10010)

32. Simpson GG. 1944 *Tempo and mode in evolution*. New York, NY: Columbia University Press.

33. Simpson GG. 1953 *The major features of evolution*. New York, NY: Columbia University Press.

34. Díaz-Fleischer F, Papaj DR, Prokopy RJ, Norrbom AL, Aluja M. 1999 Evolution of fruit fly oviposition behavior. In *Fruit flies (Tephritidae): phylogeny and evolution of behavior* (eds M Aluja, AL Norrbom), pp. 811–841. Boca Raton, FL: CRC Press.

35. Mason WRM, Huber JT. 1993 Order Hymenoptera. In *Hymenoptera of the world: an identification guide to families* (eds H Goulet, JT Huber), pp. 4–12. Ottawa, Canada: Public Works Government Services.

36. Chiu JC *et al*. 2013 Genome of *Drosophila suzukii*, the spotted wing *Drosophila*. *G3 GenesGenomesGenetics* **3**, 2257–2271. (doi:10.1534/g3.113.008185)