Bulletin of Entomological Research

cambridge.org/ber

Adaptation to different temperatures results in wing size divergence of the invading species *Drosophila nasuta* (Diptera: Drosophilidae) in Brazil

Vinícius Alcântara Carvalho Lima Santos[1], Ana Cristina Lauer Garcia[2] and Martín Alejandro Montes[1]

[1]Universidade Federal Rural de Pernambuco, Campus Dois Irmãos, Departamento de Biologia, Recife, Pernambuco, Brazil and [2]Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Vitória de Santo Antão, Pernambuco, Brazil

Research Paper

Cite this article: Santos VACL, Garcia ACL, Montes MA (2024). Adaptation to different temperatures results in wing size divergence of the invading species *Drosophila nasuta* (Diptera: Drosophilidae) in Brazil. *Bulletin of Entomological Research* 1–7. https://doi.org/10.1017/S0007485324000580

Received: 8 February 2024
Revised: 23 July 2024
Accepted: 14 September 2024

Keywords:
abiotic factors; Brazilian biomes; exotic species; insects; microevolution; morphometry

Corresponding author:
Ana Cristina Lauer Garcia;
Email: anacristina.garcia@ufpe.br;
Martín Alejandro Montes;
Email: martin.montes@ufrpe.br

Abstract

Invasive species threaten biodiversity on a global scale. The success of invasions depends on the species' adaptation to the different environmental conditions of new territories. Studies show that invasive insects present evolutionary changes in wing morphology in areas they are introduced to in response to abiotic conditions. In the last decade, the Asian *Drosophila nasuta* fly invaded and spread widely throughout Brazil. This insect has preferences for conserved environments and is related to the likely reduction in the abundance of native drosophilids in the Atlantic Forest. Ecological niche modelling analyses showed that rainfall and temperature are the main factors which delimit the geographic distribution of this species. Herein, we verified the existence of significant differences in the wing sizes of *D. nasuta* in Brazil and evaluated the influence of abiotic factors (rainfall and temperature) on the observed patterns. We conducted 11 measurements on the right-side wings of 240 *D. nasuta* males collected in the Amazon Forest, *Caatinga*, *Cerrado* and Atlantic Forest. Statistical analyses revealed the existence of two groups: one with larger wings, which brought together samples from locations with the lowest temperatures; and one with smaller wings, which corresponded to places with a hotter climate. One explanation for this result is the fact that large wings favour greater heat capture by flies in colder climates, increasing their survival chances in these environments. These rapid evolutionary changes in *D. nasuta* in this first decade of invasion in Brazil reveal the enormous adaptive potential of this species in this megadiverse country.

Introduction

Biological invasions cause global changes due to their impacts on ecosystems and biodiversity (Pyšek *et al.*, 2020), being one of the main threats to species extinctions (Mollot *et al.*, 2017; Spatz *et al.*, 2023; Su *et al.*, 2023). International trade, transport and tourism have contributed to an exponential increase in invasive species worldwide in recent decades (Seebens *et al.*, 2018; Sun *et al.*, 2024). Invasive species must present adaptive responses to different selective pressures faced in the environments where they are introduced in order to ensure their survival and colonisation of new habitats (Schäfer *et al.*, 2018). Studies on rapid evolutionary changes in invasive species are of great interest because they can identify phenotypic characteristics which favour dispersal and successful colonisation of new habitats (Rejmánek and Richardson, 1996).

Evolutionary studies with invasive insect species have widely used wing morphology (Huey *et al.*, 2000; Gilchrist *et al.*, 2004; Loh *et al.*, 2008; Laparie *et al.*, 2016). This is an attractive structure for this kind of investigation, since wings are of wide importance in a variety of aspects of an insect's life, such as reproduction, territorial display, foraging, defence mechanisms, thermal regulation and aerodynamics (Bettsand and Wootton, 1988; Wootton, 1992; Berwaerts *et al.*, 2006; Pass, 2018). Changes in environmental conditions are capable of promoting quantitative variations in physiology and morphology of insect wings. Reducing the frequency of wingbeats at high temperatures is an adaptive strategy in bees which favours thermoregulation (Glass *et al.*, 2024). Several studies on the Drosophilidae family have demonstrated that abiotic parameters (such as temperature) influence the wing size of invasive species, with flies developing larger wings as an adaptive response to occupying environments with lower temperatures (Karan *et al.*, 1999; Huey *et al.*, 2000; Gilchrist *et al.*, 2004; Gilchrist and Huey, 2004; Loh *et al.*, 2008).

The Asian *Drosophila nasuta* (Diptera: Drosophilidae) fly is an invasive species in Brazil with a notable ability to expand its geographic distribution in this area of introduction (Martins *et al.*, 2023). The first record of this species in Brazil occurred in the *Cerrado* (savanna), approximately 10 years ago (Leão *et al.*, 2017). Since then, *D. nasuta* has spread throughout different Brazilian biomes, such as the Atlantic Forest (Vilela and Goñi, 2015; Silva *et al.*, 2020), the *Caatinga* (xeric shrubland) (Montes *et al.*, 2021), the Amazon Forest (Medeiros *et al.*, 2022) and the Pantanal wetlands (Martins *et al.*, 2023). The species currently occupies more than half of Brazil's area (Martins *et al.*, 2023). Genetic studies in Brazil indicate population structuring of *D. nasuta* (Santos *et al.*, 2021), reflecting its evolutionary potential in this territory. Some possible effects of ecosystem imbalances caused by *D. nasuta* in Brazil have been observed in the north of the Atlantic Forest, where a reduction in the abundance of native drosophilids was reported after this species arrived (Oliveira, 2021). Furthermore, *D. nasuta* shows a preference for preserved environments compared to anthropised areas (Silva *et al.*, 2020), which represents a threat to the biodiversity of invaded territories.

Ecological niche modelling data projected the geographic expansion of *D. nasuta* in different invasion areas, especially in conservation units in Central and South America. These data also indicated that rainfall and temperature parameters are mainly responsible for limiting the global distribution of this species (Garcia *et al.*, 2022).

In this work, significant differences in the wing size of Brazilian *D. nasuta* populations obtained in Amazon Forest, *Caatinga*, *Cerrado* and Atlantic Forest areas were evaluated. The influence of abiotic factors known to be important for the geographic distribution of *D. nasuta*, such as rainfall and temperature, were tested to understand the geographical pattern of the observed morphological variation.

Materials and methods

Drosophila nasuta *sampling locations and capture method*

Drosophilids were collected in Brazil in areas within the Amazon Forest, *Caatinga*, *Cerrado* and Atlantic Forest biomes (fig. 1). Sampling was always performed during periods of greater rainfall in the areas investigated between 2019 and 2021 in order to remove the morphological variation associated with seasonality (Przybylska *et al.*, 2016) (table 1).

The biomes studied are areas where *D. nasuta* has been recorded in greater abundance in South America (Leão *et al.*, 2017; Silva *et al.*, 2020; Montes *et al.*, 2021; Martins *et al.*, 2023), representing a wide territory of its distribution on this continent (Martins *et al.*, 2023). The Amazon is the largest tropical rainforest in the world, covering nine countries in South America, with 59% of its area in Brazil (IBGE, 2019). It is one of the biomes with the greatest biodiversity on the planet (Guayasamin *et al.*, 2024). The *Caatinga* is the largest and most diverse seasonally dry tropical forest in the world, occurring exclusively in Brazil, where it occupies around 10% of its territory (Silva *et al.*, 2017; IBGE, 2019). The *Cerrado* is the most biodiverse savanna on the planet, extending across three countries (Brazil, Paraguay and Bolivia), but mainly found in Brazil where it occupies 24% of its territory (Walter *et al.*, 2008; IBGE, 2019). This biome is recognised as one of the hotspots for conservation (Mittermeier *et al.*, 2011). The Atlantic Forest occupies approximately 13% of the Brazilian territory. It is mainly distributed along its coast, also extending to part of Argentina and Paraguay (IBGE, 2019). It is one of the richest humid tropical forests in the world, featuring many endemic and endangered species, and is one of the world's hotspots (Tabarelli *et al.*, 2005).

Drosophilids were sampled in each of the eight investigated locations (table 1), using ten traps made from plastic bottles containing banana bait (Tidon and Sene, 1988). The traps were suspended 1.5 m from the ground and distributed randomly (at a minimum distance of 30 m between them) and 50 m away from the edges of the forest fragments where they remained exposed for three consecutive days. The captured drosophilids were stored in 70% ethanol and the *D. nasuta* specimens were identified according to Vilela and Goñi (2015) by their light body colour,

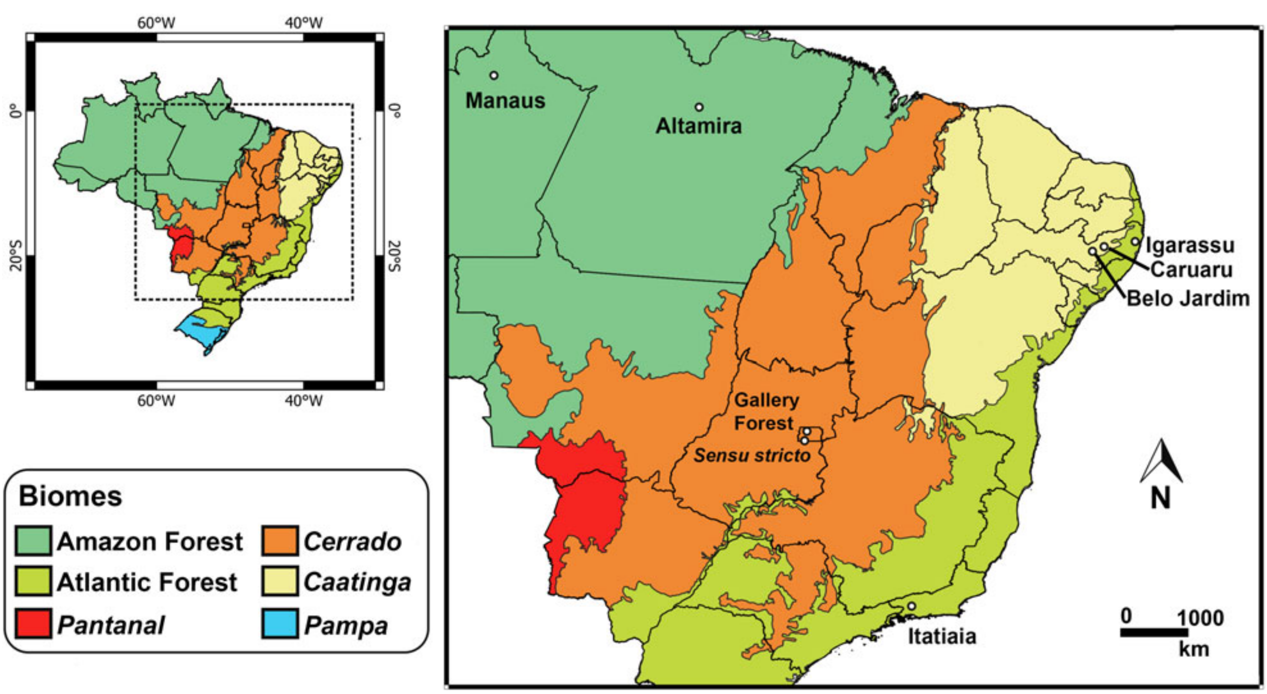

Figure 1. On the left, map of Brazil with an indication of its biomes. On the right, partial enlargement of the map, indicating the sampling locations of *Drosophila nasuta*.

https://doi.org/10.1017/S0007485324000580 Published online by Cambridge University Press

Table 1. *Drosophila nasuta* biomes and sampling locations in Brazil with data on geographic coordinates, sampling dates and climate characterisation (temperature and rainfall)

Biomes	Locations	Codes	Coordinates	Sample date	Mean maximum annual temperature (°C)	Mean minimum annual temperature (°C)	Annual rainfall (mm)
Amazon Forest	Altamira National Forest	Altamira	4°21′S/52°25′W	January/2020	30.8[a]	23.2[a]	1904[a]
	Federal University of Amazonas	Manaus	3°06′S/59°58′W	January/2021	30.7[b]	23.8[b]	2931[b]
Caatinga	Experimental Station of the Agronomic Institute of Pernambuco	Caruaru	8°14′S/35°55′W	July/2019	28.0[c]	19.7[c]	572[c]
	Bituri Farm Private Natural Heritage Reserve	Belo Jardim	8°14′S/36°22′W	July/2019	28.4[d]	18.6[d]	428[d]
Cerrado	Ecological Station of the Brasília Botanical Garden	*Sensu stricto*	15°54′S/47°52′W	January/2020	27.1[e]	17.1[e]	1502[e]
		Gallery Forest	15°53′S/47°49′W	January/2020	27.1[e]	17.1[e]	1502[e]
Atlantic Forest	Charles Darwin Ecological Refuge	Igarassu	7°48′S/34°57′W	July/2019	28.4[f]	23.0[f]	965[f]
	Itatiaia National Park	Itatiaia	22°26′S/44°37′W	January/2020	24.4[g]	15.5[g]	2547[g]

The codes for the locations are the same as those used in fig. 1.
[a]Climatempo (2024a) Climatologia histórica de Altamira, Pará. https://www.climatempo.com.br/climatologia/228/altamira-br (Accessed 20 January 2024).
[b]Climatempo (2024b) Climatologia histórica de Manaus, Amazonas. https://www.climatempo.com.br/climatologia/25/manaus-am (Accessed 20 January 2024).
[c]Climatempo (2024c) Climatologia histórica de Caruaru, Pernambuco. https://www.climatempo.com.br/climatologia/764/caruaru-pe (Accessed 20 January 2024).
[d]Climatempo (2024d) Climatologia histórica de Belo Jardim, Pernambuco. https://www.climatempo.com.br/climatologia/2179/belojardim-pe (Accessed 20 January 2024).
[e]Climatempo (2024e) Climatologia histórica de Brasília, Distrito Federal. https://www.climatempo.com.br/climatologia/61/brasilia-df (Accessed 20 January 2024).
[f]Climatempo (2024f) Climatologia histórica de Igarassu, Pernambuco. https://www.climatempo.com.br/climatologia/1256/igarassu-pe (Accessed 20 January 2024).
[g]Climatempo (2024g) Climatologia histórica de Itatiaia, Rio de Janeiro. https://www.climatempo.com.br/climatologia/303/itatiaia-rj (Accessed 20 January 2024).

the presence of a longitudinal brown stripe in the middle dorsal area of the pleura, a silvery and whitish fringe in the head region when viewed from the front, a row of cuneiform setae on the anteroventral side of the femur on the forelegs, wings with a costal index of about 3.1, and male terminalia characteristics.

Morphometric and statistical analyses

The *D. nasuta* individuals collected were separated by sex and geographic origin. Separation by sex was performed by analysing the flies' terminalia, with males being distinguished from females by the presence of an aedeagus and hypandrium and the absence of an ovipositor. Flies were discarded if they had torn or wrinkled wings. A total of 30 male individuals from each population were dissected with a 70% ethanol solution. The right-side wing of each individual was removed by squeezing the wing joint with tweezers and pulling the wing away from the body, using a pair of tweezers to hold the body in place. Only the right-side wings were used to avoid fluctuating asymmetry variations.

The dissected wings were placed on microscope slides with the ventral side facing down and covered with a 1:1 solution of absolute ethanol and glycerol. Slides were covered with coverslips and any air bubbles were gently removed by pressing the coverslip with forceps. The wings were digitally photographed on an Instrutherm MBB-200 microscope at $40\times$ magnification.

Next, 11 measurements were taken from the digitised images on each wing from reference points at the junction or termination of the venations, following the parameters of Bitner-Mathé and Klaczko (1999) (fig. 2). Measurements were performed using the tpsDIG program (Rohlf, 2016). The wings of all specimens were mounted, photographed and measured by the same person in order to minimise possible errors in morphometric analyses, in accordance with the recommendations of Fox *et al.* (2020).

Arithmetic means and standard deviations were obtained for each of the 11 wing measurements for samples from different geographic locations. Analysis of variance (ANOVA) was performed with the Tukey *a posteriori* test to observe possible differences in wing measurements between locations. The wing measurements of individuals from locations which did not show statistical differences in previous tests were grouped. The established groups were analysed using a linear discriminant function. Pearson's correlation test was performed between wing measurements and abiotic factors (rainfall and maximum and minimum temperatures). All of these analyses were carried out using the PAST version 4.3 program (Hammer *et al.*, 2001) and a significance level of $P < 0.05$ or $P < 0.001$ was used in the statistical tests.

Results

The lowest averages for the 11 wing measurements evaluated in 240 *D. nasuta* individuals were observed for the populations of the Amazon Forest (Altamira and Manaus) and the north of the Atlantic Forest (Igarassu). The *Caatinga* (Caruaru and Belo Jardim), the *Cerrado* (*sensu strictu* and Gallery Forest) and the south of the Atlantic Forest (Itatiaia) populations presented the highest averages for these measurements (table 2).

The two groups observed by analysing the mean *D. nasuta* wing measurements were also verified by ANOVA and the subsequent Tukey's test (table 3, Supplementary table 1). One of the groups was formed by populations from the Amazon Forest (Altamira and Manaus) and the north of the Atlantic Forest (Igarassu), with no significant difference between these samples. Another group brought together populations from the *Caatinga* (Caruaru and Belo Jardim), the *Cerrado* (*sensu strictu* and Gallery Forest) and the south of Atlantic Forest (Itatiaia), also without significant differences between the wing measurements of these populations. Comparisons of wing measurements

https://doi.org/10.1017/S0007485324000580 Published online by Cambridge University Press

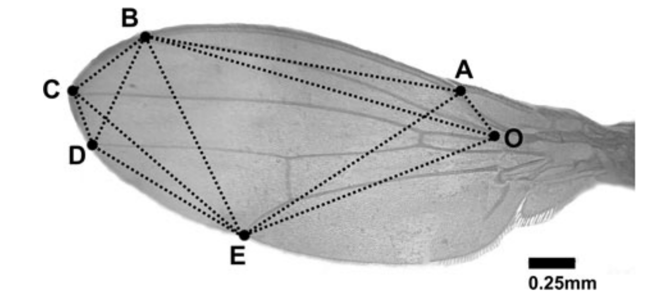

Figure 2. Right wing of a male *Drosophila nasuta* with indications of the measurements that were taken from six reference points: OA, OB, OE, AB, AE, BC, BD, BE, CD, CE and DE.

between the populations of these two groups showed significant differences ($P < 0.001$) (table 3).

The groupings formed in the previous analyses were evaluated using a linear discriminant function, which confirmed the existence of these two distinct groups. In this analysis, 91.67% of individuals were correctly identified in their corresponding groups by the cross-validation test (table 4).

The group of individuals with the smallest wings coincided with the locations with the highest maximum and minimum temperatures detected. The group with the largest wings corresponded to the areas with the lowest maximum and minimum temperatures (table 1). A high negative and significant correlation was observed between maximum temperatures and four of the 11 wing measurements investigated, as well as for all wing measurements and minimum temperatures. No significant correlation regarding rainfall was observed with any of the wing measurements analysed (table 5).

Discussion

The Asian *D. nasuta* fly invaded Brazil approximately 10 years ago (Leão *et al.*, 2017). The species has already expanded over an area of 4.6 million km^2 in this short period, which corresponds to 55% of the Brazilian territory (Martins *et al.*, 2023). Data from 11 wing measurements taken on 240 individuals of this species from different Brazilian biomes in the present study revealed statistically significant differences between the geographic samples.

The variations in wing sizes observed herein resulted in forming two groups of *D. nasuta*. Other invasive drosophilids in the Neotropical region also showed significant differences in wing morphology in different areas of introduction. Loh and Bitner-Mathé (2005) detected variations in the wing size and shape of the African *Zaprionus indianus* fly in areas recently invaded by the species in Brazil. Some authors have observed significant differences in the morphometry of drosophilid wings in comparison with invaded areas, and in comparing these areas with locations where the species are native; for example, in studies conducted with *Z. indianus* (David *et al.*, 2006; Yassin *et al.*, 2009) and *D. suzukii* (Fraimout *et al.*, 2018). Taken together, our results and those of these investigations reveal the capacity for morphological differentiation in the wings of invasive drosophilids in introduced areas.

Drosophila nasuta individuals with larger wings were observed in locations with colder temperature extremes (*Caatinga*, *Cerrado* and south of Atlantic Forest) and those with smaller wings occurred in locations with higher minimum and maximum temperature extremes (Amazon Forest and north of the Atlantic Forest). Changes in environmental temperature conditions are

Table 2. Arithmetic means (in mm) and standard deviations for the different reference point measurements of the right wings of *Drosophila nasuta* males from different biomes and locations in Brazil

		Wing measurements										
Biomes	Locations	OA	OB	OE	AB	AE	BC	BD	BE	CD	CE	DE
Amazon Forest	Altamira	0.22 ± 0.02	1.36 ± 0.06	1.03 ± 0.05	1.21 ± 0.05	1.03 ± 0.06	0.36 ± 0.02	0.48 ± 0.02	0.87 ± 0.04	0.2 ± 0.01	0.85 ± 0.04	0.69 ± 0.03
	Manaus	0.21 ± 0.02	1.37 ± 0.07	1.03 ± 0.05	1.22 ± 0.06	1.02 ± 0.05	0.37 ± 0.02	0.49 ± 0.02	0.88 ± 0.04	0.21 ± 0.02	0.87 ± 0.04	0.7 ± 0.04
Caatinga	Caruaru	0.24 ± 0.02	1.6 ± 0.07	1.19 ± 0.05	1.42 ± 0.07	1.17 ± 0.05	0.4 ± 0.02	0.54 ± 0.02	1.00 ± 0.05	0.23 ± 0.03	0.97 ± 0.05	0.78 ± 0.04
	Belo Jardim	0.23 ± 0.02	1.57 ± 0.08	1.17 ± 0.06	1.41 ± 0.08	1.16 ± 0.06	0.4 ± 0.03	0.54 ± 0.03	0.99 ± 0.05	0.24 ± 0.01	0.97 ± 0.04	0.78 ± 0.04
Cerrado	*Sensu stricto*	0.24 ± 0.02	1.55 ± 0.06	1.17 ± 0.04	1.37 ± 0.06	1.14 ± 0.04	0.38 ± 0.02	0.52 ± 0.02	0.97 ± 0.04	0.23 ± 0.02	0.95 ± 0.05	0.77 ± 0.04
	Gallery Forest	0.24 ± 0.02	1.58 ± 0.06	1.19 ± 0.05	1.41 ± 0.05	1.18 ± 0.05	0.4 ± 0.02	0.54 ± 0.03	0.99 ± 0.04	0.23 ± 0.01	0.96 ± 0.04	0.78 ± 0.03
Atlantic Forest	Igarassu	0.22 ± 0.01	1.4 ± 0.07	1.06 ± 0.05	1.24 ± 0.07	1.04 ± 0.05	0.37 ± 0.02	0.49 ± 0.03	0.89 ± 0.05	0.21 ± 0.02	0.87 ± 0.05	0.7 ± 0.04
	Itatiaia	0.24 ± 0.02	1.55 ± 0.09	1.16 ± 0.06	1.38 ± 0.08	1.14 ± 0.06	0.4 ± 0.03	0.53 ± 0.03	0.97 ± 0.05	0.23 ± 0.02	0.96 ± 0.05	0.77 ± 0.04

The reference points for wing measurements are illustrated in fig. 2.

https://doi.org/10.1017/S0007485324000580 Published online by Cambridge University Press

Table 3. Tukey's test ($P < 0.001$) for measurements of the right wings of *Drosophila nasuta* males collected in different locations in Brazilian biomes

	Wing measurements										
Locations	OA	OB	OE	AB	AE	BC	BD	BE	CD	CE	DE
Altamira × Manaus	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Altamira × Caruaru	*	*	*	*	*	*	*	*	*	*	*
Altamira × Belo Jardim	ns	*	*	*	*	*	*	*	*	*	*
Altamira × *Sensu stricto*	*	*	*	*	*	*	*	*	*	*	*
Altamira × Gallery Forest	*	*	*	*	*	*	*	*	*	*	*
Altamira × Igarassu	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Altamira × Itatiaia	*	*	*	*	*	*	*	*	*	*	*
Manaus × Caruaru	*	*	*	*	*	*	*	*	*	*	*
Manaus × Belo Jardim	*	*	*	*	*	*	*	*	*	*	*
Manaus × *Sensu stricto*	*	*	*	*	*	ns	*	*	*	*	*
Manaus × Gallery Forest	*	*	*	*	*	*	*	*	*	*	*
Manaus × Igarassu	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Manaus × Itatiaia	*	*	*	*	*	*	*	*	*	*	*
Caruaru × Belo Jardim	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Caruaru × *Sensu stricto*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Caruaru × Gallery Forest	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Caruaru × Igarassu	*	*	*	*	*	*	*	*	*	*	*
Caruaru × Itatiaia	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Belo Jardim × *Sensu stricto*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Belo Jardim × Gallery Forest	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Belo Jardim × Igarassu	ns	*	*	*	*	*	*	*	*	*	*
Belo Jardim × Itatiaia	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sensu stricto × Gallery Forest	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns
Sensu stricto × Igarassu	*	*	*	*	*	ns	*	*	*	*	*
Sensu stricto × Itatiaia	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Gallery Forest × Igarassu	*	*	*	*	*	*	*	*	*	*	*
Gallery Forest × Itatiaia	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Igarassu × Itatiaia	*	*	*	*	*	*	*	*	*	*	*

The codes for the locations are the same as those used in table 1, and the reference points for wing measurements are illustrated in fig. 2.
Ns, not significant. *Statistically significant.

Table 4. Classification by discriminant function analysis followed by cross-validation for the two groups obtained by analysis of means and ANOVA/Tukey's test, based on measurements of the right wings of *Drosophila nasuta* males collected in different Brazilian locations and biomes

	Group 1	Group 2	Total
Group 1	82	8	90
Group 2	12	138	150
Total	94	146	240

Group 1 = populations from the Amazon Forest (Altamira and Manaus) and the north of the Atlantic Forest (Igarassu); and Group 2 = populations from the *Caatinga* (Caruaru and Belo Jardim), the *Cerrado* (*sensu strictu* and Gallery Forest) and the south of the Atlantic Forest (Itatiaia).

recognised to promote quantitative variations in drosophilid wing morphology. As pointed out by our results, other studies have shown that invasive drosophilids have larger wings in areas with lower temperatures in places of introduction. For example, this has been observed for the European species *D. subobscura* in invaded areas in North and South America (Huey *et al*., 2000; Gilchrist *et al*., 2004; Gilchrist and Huey, 2004) and for the African species *Z. Indianus* in invaded areas in India (Karan *et al*., 1999) and South America (Loh *et al*., 2008). These authors deemed that changes in the wing size of invasive drosophilids in response to temperature variations were associated with an adaptive process.

Our results revealed a high negative correlation between wing measurements and maximum temperatures, and especially for minimum temperatures. Fraimout *et al*. (2018) tested the influence of

https://doi.org/10.1017/S0007485324000580 Published online by Cambridge University Press

Table 5. Pearson's correlation between measurements of the right wings of *Drosophila nasuta* males collected in different biomes in Brazil and abiotic factors (rainfall, maximum and minimum temperatures)

Abiotic factors	Wing measurements										
	OA	OB	OE	AB	AE	BC	BD	BE	CD	CE	DE
Rainfall	−0.20 (0.637)	−0.45 (0.261)	−0.50 (0.206)	−0.44 (0.275)	−0.45 (0.261)	−0.50 (0.208)	−0.47 (0.242)	−0.44 (0.281)	−0.44 (0.277)	−0.40 (0.327)	−0.40 (0.324)
Maximum temperatures	−0.79 (0.020*)	−0.76 (0.030*)	−0.70 (0.053)	−0.73 (0.040*)	−0.74 (0.037*)	−0.65 (0.081)	−0.69 (0.057)	−0.68 (0.062)	−0.67 (0.067)	−0.69 (0.058)	−0.66 (0.073)
Minimum temperatures	−0.79 (0.020*)	−0.88 (0.004*)	−0.84 (0.008*)	−0.89 (0.003*)	−0.88 (0.004*)	−0.72 (0.043*)	−0.88 (0.004*)	−0.86 (0.006*)	−0.84 (0.010*)	−0.86 (0.007*)	−0.83 (0.011*)

Temperature and rainfall data are shown in table 1 and wing measurements are shown in fig. 2. *P*-values are shown in parentheses. $^*P < 0.05$.

different temperatures (16, 22 and 28°C) in a laboratory on the wing morphology of the Asian species *D. suzukii* from samples collected in its area of origin in Japan and in two invasion areas, France and the United States. As observed in the present study for *D. nasuta*, the extreme minimum temperature most influenced the wing size of *D. suzukii*, resulting in individuals with larger wings compared to those at temperatures of 22 and 28°C (which did not present significant differences in wing morphology between them). The importance of minimum temperatures for the occurrence of *D. nasuta* has been highlighted by Garcia *et al.* (2022) in an ecological niche modelling study. These authors revealed that cold temperatures explain 21% of the global geographic distribution model of this species. Thus, colder minimum temperatures seem to influence the wing morphology of different drosophilid species, and at the same time, account for the geographic distribution capacity of *D. nasuta*.

Why were the largest wings of *D. nasuta* observed in individuals occupying locations with the lowest minimum temperatures? This probably occurs because large-winged insects are more effective at absorbing heat, making this trait advantageous in areas with more extreme cold conditions where obtaining and retaining heat are critical for survival (Heinrich, 1974; Douglas, 1981). Thus, the phenotypic variation found in the wings of *D. nasuta* individuals could be the result of an adaptive process related to temperature. Laboratory experiments may confirm this result by cultivating geographic samples of this species at different temperatures.

The present study is a pioneer in describing a morphological variation pattern in the wing size of *D. nasuta*, sampling individuals from a large part of the geographic distribution of this recent invasive species in Brazil. This condition reveals the adaptive potential of *D. nasuta* in introduced areas.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485324000580

Acknowledgements. This work was supported by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, process number: 425274/2018-7).

Competing interests. None.

References

Berwaerts K, Aerts P and van Dyck H (2006) On the sex-specific mechanisms of butterfly flight: flight performance relative to flight morphology, wing kinematics, and sex in *Pararge aegeria*. *Biological Journal of the Linnean Society* **89**, 675–687.

Betts CR and Wootton RJ (1988) Wing shape and flight behaviour in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. *Journal of Experimental Biology* **138**, 271–288.

Bitner-Mathé BC and Klaczko LB (1999) Size and shape heritability in natural populations of *Drosophila mediopunctata*: temporal and microgeographical variation. *Genetica* **105**, 35–42.

David JR, Araripe LO, Bitner-Mathé BC, Capy P, Goñi B, Klaczko LB, Legout H, Martins MB, Voudibio J, Yassin A and Moreteau B (2006) Quantitative trait analysis and geographic variability of natural populations of *Zaprionus indianus*, a recent invader in Brazil. *Heredity* **96**, 53–62.

Douglas MM (1981) Thermoregulatory significance of thoracic lobes in the evolution of insect wings. *Science* **211**, 84–86.

Fox NS, Veneracion JJ and Blois JL (2020) Are geometric morphometric analyses replicable? Evaluating landmark measurement error and its impact on extant and fossil *Microtus* classification. *Ecology and Evolution* **10**, 3260–3275.

Fraimout A, Jacquemart P, Villarroel B, Aponte DJ, Decamps T, Herrel A, Cornette R and Debat V (2018) Phenotypic plasticity of *Drosophila suzukii* wing to developmental temperature: implications for flight. *Journal of Experimental Biology* **221**, jeb166868.

Garcia ACL, Silva FP, Neves CHCB and Montes MA (2022) Current and future potential global distribution of the invading species *Drosophila nasuta* (Diptera: Drosophilidae). *Biological Journal of the Linnean Society* **135**, 208–221.

Gilchrist GW and Huey RB (2004) Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. *Integrative and Comparative Biology* **44**, 461–470.

Gilchrist GW, Huey RB, Balanyà J, Pascual M, Serra L and Noor M (2004) A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. *Evolution* **58**, 768–780.

Glass JR, Burnett NP, Combes SA, Weisman E, Helbling A and Harrison JF (2024) Flying, nectar-loaded honey bees conserve water and improve heat tolerance by reducing wingbeat frequency and metabolic heat production. *Proceedings of the National Academy of Sciences* **121**, e2311025121.

Guayasamin JM, Ribas CC, Carnaval AC, Carrillo JD, Hoorn C, Lohmann LG, Riff D, Ulloa CU and Albert JS (2024) Evolution of Amazonian biodiversity: a review. *Acta Amazonica* **54**, e54bc21360.

Hammer Ø, Harper DAT and Ryan PD (2001) Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 1–9.

Heinrich B (1974) Thermoregulation in endothermic insects. *Science* **185**, 747–756.

Huey RB, Gilchrist GW, Carlson ML, Berrigan D and Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**, 308–309.

IBGE (2019) Biomas e sistema costeiro-marinho do Brasil: compatível com a escala 1: 250000. Coordenação de Recursos Naturais e Estudos Ambientais, Rio de Janeiro. Available at https://biblioteca.ibge.gov.br/visualizacao/livros/liv101676.pdf

Karan D, Moreteau B and David JR (1999) Growth temperature and reaction norms of morphometrical traits in a tropical drosophilid: *Zaprionus indianus*. *Heredity* **83**, 398–407.

Laparie M, Vernon P, Cozic Y, Frenot Y, Renault D and Debat V (2016) Wing morphology of the active flyer *Calliphora vicina* (Diptera: Calliphoridae) during its invasion of a sub-Antarctic archipelago where insect flightlessness is the rule. *Biological Journal of the Linnean Society* **119**, 179–193.

Leão BFD, Roque F, Deus PHM and Tidon R (2017) What happens when exotic species arrive in a new area? The case of drosophilids in the Brazilian Savanna. *Drosophila Information Service* **100**, 65–69.

Loh R and Bitner-Mathé BC (2005) Variability of wing size and shape in three populations of a recent Brazilian invader, *Zaprionus indianus* (Diptera: Drosophilidae), from different habitats. *Genetica* **125**, 271–281.

Loh R, David JR, Debat V and Bitner-Mathé BC (2008) Adaptation to different climates results in divergent phenotypic plasticity of wing size and shape in an invasive drosophilid. *Journal of Genetics* **87**, 209–217.

Martins TCSL, Santos MFS, Santos MMS, Araújo JS, Neves CHCB, Garcia ACL and Montes MA (2023) *Drosophila nasuta* (Diptera, Drosophilidae) in Brazil: a decade of invasion and occupation of more than half of the country. *Anais da Academia Brasileira de Ciências* **95**, e20230507.

Medeiros HF, Monteiro MP, Caçador AWB, Pereira CM, Praxedes CLB, Martins MB, Montes MA and Garcia ACL (2022) First records of the invading species *Drosophila nasuta* (Diptera: Drosophilidae) in the Amazon. *Neotropical Entomology* **51**, 493–497.

Mittermeier RA, Turner WR, Larsen FW, Brooks TM and Gascon C (2011) Global biodiversity conservation: the critical role of hotspots. In Zachos F and Habel J (eds), *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 3–22.

Mollot G, Pantel JH and Romanuk TN (2017) The effects of invasive species on the decline in species richness: a global meta-analysis. *Advances in Ecological Research* **56**, 61–83.

Montes MA, Neves CHCB, Ferreira AF, Santos MFS, Quintas JIFP, Manetta GDA, Oliveira PV and Garcia ACL (2021) Invasion and spreading of *Drosophila nasuta* (Diptera, Drosophilidae) in the Caatinga Biome, Brazil. *Neotropical Entomology* **50**, 571–578.

Oliveira GH (2021) Avaliação da homogeneização biótica e preferência ambiental de drosofilídeos invasores no norte da Floresta Atlântica (Masters Thesis). Universidade Federal Rural de Pernambuco, Brazil.

Pass G (2018) Beyond aerodynamics: the critical roles of the circulatory and tracheal systems in maintaining insect wing functionality. *Arthropod Structure and Development* **47**, 391–407.

Przybylska MS, de Brito FA and Tidon R (2016) Ecological insights from assessments of phenotypic plasticity in a Neotropical species of *Drosophila*. *Journal of Thermal Biology* **62**, 7–14.

Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ and Richardson DM (2020) Scientists' warning on invasive alien species. *Biological Reviews* **95**, 1511–1534.

Rejmánek M and Richardson DM (1996) What attributes make some plant species more invasive? *Ecology* **77**, 1655–1661.

Rohlf FJ (2016) Thin-plate spline (TPS) computer program. Available at http://www.sbmorphometrics.org/index.html

Santos MFS, Neves CHCB, Oliveira E, Ribeiro MC, Faria-Júnior JEQ, Montes MA and Garcia ACL (2021) Genetic diversity of the invasive species *Drosophila nasuta* (Diptera, Drosophilidae) in different biomes in Brazil. p. 275. In: 66° Brazilian Congress of Genetics, Ribeirão Preto, Brazil, 13 September – 16 September, Brazilian Society of Genetics.

Schäfer MA, Berger D, Rohner PT, Kjaersgaard A, Bauerfeind SS, Guillaume F, Fox CW and Blanckenhorn WU (2018) Geographic clines in wing morphology relate to colonization history in New World but not Old World populations of yellow dung flies. *Evolution* **72**, 1629–1644.

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, van Kleunen M, Winter M, Ansong M, Arianoutsou M, Bacher S, Blasius B, Brockerhoff EG, Brundu G, Capinha C, Causton CE, Celesti-Grapow L, Dawson W, Dullinger S, Economo EP, Fuentes N, Guénard B, Jäger H, Kartesz J, Kenis M, Kühn I, Lenzner B, Liebhold AM, Mosena A, Moser D, Nentwig W, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, Walker K, Ward DF, Yamanaka T and Essl F (2018) Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences* **115**, E2264–E2273.

Silva JMC, Leal IR and Tabarelli M (2017) *Caatinga: The Largest Tropical Dry Forest Region in South America*. New York: Springer.

Silva DG, Schmitz HJ, Medeiros HF, Rohde C, Montes MA and Garcia ACL (2020) Geographic expansion and dominance of the invading species *Drosophila nasuta* (Diptera, Drosophilidae) in Brazil. *Journal of Insect Conservation* **24**, 525–534.

Spatz DR, Jones HP, Bonnaud E, Kappes P, Holmes ND and Guzmán YB (2023) Invasive species threats to seabirds. In Young L and VanderWerf E (eds), *Conservation of Marine Birds*. London: Academic Press, pp. 97–130.

Su T, Cui G, Man Z, Li W, Huang Z, Chen J and Zhao M (2023) Interspecific association of sika deer in terrestrial animal communities of Liancheng National Nature Reserve, China. *Integrative Zoology* **18**, 688–703.

Sun J, Koski TM, Wickham JD, Baranchikov YN and Bushley KE (2024) Emerald ash borer management and research: decades of damage and still expanding. *Annual Review of Entomology* **69**, 239–258.

Tabarelli M, Pinto LP, Silva JM, Hirota M and Bede L (2005) Challenges and opportunities for biodiversity conservation in the Brazilian Atlantic Forest. *Conservation Biology* **19**, 695–700.

Tidon R and Sene FM (1988) A trap that retains and keeps *Drosophila* alive. *Drosophila Information Service* **67**, 89.

Vilela CR and Goñi B (2015) Is *Drosophila nasuta* Lamb (Diptera, Drosophilidae) currently reaching the status of a cosmopolitan species? *Revista Brasileira de Entomologia* **59**, 346–350.

Walter BMT, Carvalho AD and Ribeiro JF (2008) O conceito de savana e de seu componente *Cerrado*. In Sano SM, de Almeida SP and Ribeiro JF (eds), *Cerrado*: ecologia e flora. Distrito Federal: Embrapa Cerrados, pp. 21–45.

Wootton RJ (1992) Functional morphology of insect wings. *Annual Review of Entomology* **37**, 113–140.

Yassin A, David JR and Bitner-Mathé BC (2009) Phenotypic variability of natural populations of an invasive drosophilid, *Zaprionus indianus*, on different continents: comparison of wild-living and laboratory-grown flies. *Comptes Rendus Biologies* **332**, 898–908.