



Wing morphology of the active flyer *Calliphora vicina* (Diptera: Calliphoridae) during its invasion of a sub-Antarctic archipelago where insect flightlessness is the rule

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The cosmopolitan blowfly *Calliphora vicina* became established in the sub-Antarctic Kerguelen Islands in the late 1970s, following a warming period that allowed its full development. Although temperature and wind may limit flight activity, the fly invaded the archipelago, reaching sites remote from the introduction point. Most native competitors have converged to flightlessness as a response to stringent environmental conditions and therefore the flight strategy of *C. vicina* might be either a handicap or a competitive advantage under ongoing climate change. Using geometric morphometrics, we investigated whether the wing had changed over time in *C. vicina* within the archipelago (1998 vs. 2009) and compared its morphology with that of a continental population from a temperate area (1983 vs. 2009). Wing shape plasticity to temperature was also experimentally investigated. We found no clues of relaxed selection on flight morphology in the range invaded. However, rapid changes of wing shape occurred over time in females from the Kerguelen Islands compared with both males and females of the continental population, despite a shorter time-lag between samples in the former. The thermal reaction norms for wing shape found for *C. vicina* from Kerguelen were also different from those of the continental population, but it remains unknown whether this resulted from or preceded the introduction. These combined findings are consistent with a fingerprint of local adaptation in the invasive population. However, the adaptive significance of the changes, in terms of their aerodynamic consequences and the future evolution of *C. vicina* in the Kerguelen Islands, requires further investigation. From an evolutionary standpoint, sustaining flight capability under the novel sub-Antarctic conditions might be critical to the invasive success of *C. vicina* as most competitors are flightless. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

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INTRODUCTION

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Active flight is a rapid and energy-efficient mode of dispersal that triggers the adaptive radiation of insects (Dickinson, Lehmann & Sane, 1999; Dudley,

2002). In some instances, however, the physical environment may prevent flight or make long-range dispersal useless (e.g. in the case of subterranean insects). Thus, wing reduction or winglessness is found in many insect species, presumably resulting from the counter-selection of wing development because of trade-offs with other biological functions (Roff, 1986, 1990; Wagner & Liebherr, 1992). The example of insects from the sub-Antarctic Islands is particularly striking. Within the Kerguelen archipelago, 19 of the 23 native insect species have evolved flightlessness or even winglessness, including some native brachyceran and nematoceran flies, and also insects from other orders, such as a moth (Schermann-Legionnet *et al.*, 2007). Consistently, the only native winged species are weak fliers (Schermann-Legionnet *et al.*, 2007), such as the polymorphous fly *Listriomastax litorea* (Diptera, Tethinidae), which has three morphs but only one macropterous form compatible with flight (the other forms are brachypterous and micropterous; Séguy, 1965).

This evolutionary convergence to flightlessness in native sub-Antarctic insects has been associated with the combination of several environmental factors (Gressitt & Weber, 1959; Vernon, 1981). First, the strong winds frequently encountered in the sub-Antarctic islands (Vernon, 1981; Chevrier, 1996) may shorten possible flight windows (see Taylor, 1963). Second, flying activity requires a high metabolic rate that may not be met under the year-round cool temperatures and buffered climate of these areas [e.g. on Kerguelen Islands over the period 1951–1980, the annual temperature was (mean \pm SD) $4.4 \pm 0.9^\circ\text{C}$, and the temperature from February to July ranged from $7.4 \pm 0.9^\circ\text{C}$ to $1.9 \pm 0.8^\circ\text{C}$; Lebouvier *et al.*, 2011]. Meanwhile, temperatures up to 20°C can occur, but this is rare (source: Météo France). Third, the selective pressures for long-range dispersal may be lowered in native saprophagous invertebrates, which represent the dominant feeding guild (Lebouvier *et al.*, 2011), as they mostly complete their development along the coast where large amounts of trophic resources are supplied by breeding colonies of marine vertebrates (i.e. penguins and seals) (Vernon, 1981; Vernon, Vannier & Tréhen, 1998). Fourth, the very low native predation pressures on terrestrial invertebrates (Chevrier, Vernon & Frenot, 1997; Laparie *et al.*, 2010; Lebouvier *et al.*, 2011) may reduce the need for rapid locomotion and escape. The benefits of wing development and flight may thus have been overruled by the associated costs, leading to life strategies based on flightlessness or winglessness. As an example, relaxed flight constraints enabled physogastry and even loss of flight muscles in the native fly *Anatalanta aptera* (Diptera, Sphaeroceridae), increasing the space available for

abdominal and thoracic reserves and strongly enhancing starvation resistance during austral winters when most vertebrate colonies have left (Vernon, 1981, 1986). Yet, this low diversity of insect trophic, reproductive, and dispersal strategies has increased during the 20th century as a result of species introductions associated with human activities (see Chown, Gremmen & Gaston, 1998; Frenot *et al.*, 2001, 2005; Chown, Lee & Shaw, 2008; Lebouvier *et al.*, 2011). Of the 26 alien arthropod species established in the Kerguelen Islands, six have become invasive (Frenot *et al.*, 2005). Their successful establishment and spread may rely on biological adjustments to novel abiotic (e.g. climate) and biotic (e.g. species interactions) conditions (Huey, Gilchrist & Hendry, 2005; Facon *et al.*, 2006; Davis, 2009). The question is thus raised of whether (and, if so, how) these invaders changed to succeed under the distinctive conditions of the sub-Antarctic islands.

The invasion of the blue blowfly *Calliphora vicina* Robineau-Desvoidy 1830 (Diptera, Calliphoridae) offers a unique opportunity to investigate such real-time changes in ecological and evolutionary processes under novel selection regimes (Chevrier *et al.*, 1997; Huey *et al.*, 2005; Sax *et al.*, 2007; Lebouvier *et al.*, 2011). The naturalization of this active flyer was reported in 1978 at the research station of the Kerguelen Islands (Chevrier *et al.*, 1997). It was probably transported by ship and subsequently benefitted from warming air temperatures in this region in the 1970s (see the meteorological data in Lebouvier *et al.*, 2011). Similarly to native flies, *C. vicina* has a saprophagous diet and has become one of their most common food-competitors; however, it exhibits a distinctive dispersal strategy as it is the most active insect flyer in the Kerguelen Islands (Schermann-Legionnet *et al.*, 2007). Interestingly, the combination of its long-range flight dispersal and *r* reproductive strategy is new to the guild of decomposers on these islands, and there is no competitor to match its ability in rapidly reaching remote food patches (Chevrier *et al.*, 1997). Taking advantage of the situation offered by this recent invasion, we questioned whether the blue bottle fly benefits from its flight capability in sub-Antarctic ecosystems or is susceptible to a ‘sub-Antarctic flightlessness syndrome’. In other words, have *C. vicina*’s flight characteristics changed over time and can we track such an evolutionary trend?

Using landmark-based geometric morphometrics, we tested if any peculiar evolution of the size and shape of the wing of *C. vicina* could be detected in flies from the Kerguelen Islands over time. As the geographical origin of *C. vicina* introduced in the Kerguelen Islands is unknown, we used a population from a native area (Brittany, France) as a control

with which to compare wing change over time. Wings of flies trapped in 2009 at the two locations were compared with flies collected in the same places, 11 (in the Kerguelen Islands in 1998) and 26 (in Brittany in 1983) years earlier. Given the particular Kerguelen climate (low seasonal variations with a narrow thermal range; Lebouvier *et al.*, 2011) and the expected thermal plasticity of wing shape in insects (Imasheva, Moreteau & David, 2000; Debat *et al.*, 2003, 2008), we also compared thermal plasticity between the two populations. This was undertaken by rearing separate broods of individuals trapped in 2009 in the Kerguelen Islands and in Brittany under a range of temperatures spanning 4 °C to 20 °C. We hypothesized that: (1) the wing morphology changed more in the Kerguelen Islands than in a native continental region (Brittany) as a result of stringent environmental conditions; (2) selective pressures on flight in the Kerguelen Islands may alter wing size, variability, or morphological changes in functional regions of the wing; and (3) thermal plasticity of the wing differs among populations.

MATERIAL AND METHODS

SAMPLE COLLECTION

As a part of a long-term monitoring programme conducted in the Kerguelen Islands, wild adults of *C. vicina* were sampled using Upton-type traps in the vicinity of the Port-aux-Français research station (Kerguelen Islands, France: 49°21'00"S, 70°13'00"E) during the austral summers of 1998 (February and March) and 2009 (January and February). Individuals of *C. vicina* from a temperate continental region in a native area were sampled in the vicinity of the Paimpont biological research station (Brittany, metropolitan France: 48°00'13"N, 2°13'42"W) during

the summers of 1983 (June and July) and 2009 (August). Monthly mean shade temperatures during trapping sessions in each location are detailed in Table 1. We grouped trapping sessions in periods not exceeding 2 months to constitute the 'past' and 'recent' samples that were used in the present study for allochronic comparisons. There was a gap of 11 years between the two trapping sessions in the Kerguelen Islands, and a gap of 26 years for those in Brittany. Flies used for measurements were stored in 70% ethanol.

THERMAL PHENOTYPIC PLASTICITY

At both locations, individuals trapped in 2009 were placed in plastic boxes (20 ± 0.5 °C, 70% relative humidity, photoperiod of 16:8 L:D) and supplied with pieces of sheep liver *ad libitum* to stimulate reproduction and egg-laying. After egg-laying, the adults were immediately placed in 70% ethanol before measurements were made. Batches of 30 eggs were randomly placed onto pieces of sheep liver supplied in excess in plastic boxes. Each box was then transferred directly to an incubator set at one of the following five temperatures: 4, 8, 12, 16, and 20 (± 0.5) °C. As our aim was to identify the thermal reaction norms of *C. vicina* by maximizing shape differences, we selected this temperature range as it is the broadest ecologically relevant range that we could investigate within the field campaign (lower temperatures are below the developmental threshold and higher temperatures usually do not occur on Kerguelen Islands). Hatching did not occur at 4 °C before the end of the field campaign at the Kerguelen Islands, as this temperature is close to the lower developmental limit for *C. vicina* (Davies & Ratcliffe, 1994; Vinogradova, 2009). After completion of development, emergence, and wing opening, the resultant adults reared at 8,

Table 1. Details of trapping sessions and associated monthly mean shade temperatures (source: Météo France records)

Population	Trapping session	Min, °C	Max, °C	Mean, °C
Kerguelen	February 1998 (past)	5.4 (0.4)	11.9 (0.6)	8.1 (0.5)
	March 1998 (past)	4.0 (0.4)	11.3 (0.5)	7.4 (0.4)
	January 2009 (recent)	3.8 (0.3)	10.3 (0.5)	6.7 (0.3)
	February 2009 (recent)	4.8 (0.4)	11.9 (0.5)	8.1 (0.4)
Brittany	June 1983 (past)	9.2 (0.6)	20.8 (0.8)	15.0 (0.8)
	July 1983 (past)	14.5 (0.5)	26.0 (0.8)	20.2 (0.7)
	August 2009 (recent)	11.4 (0.6)	23.2 (0.6)	17.3 (0.4)

Values are given as mean (standard error). The trapping sessions used to constitute the 'past' and 'recent' samples of each population, subsequently compared in this study, did not exceed 2 months.

Max, average temperature computed from the daily maxima; Mean, average temperature computed from the daily means; Min, average temperature computed from the daily minima.

12, 16, and 20 °C were stored in 70% ethanol until required for measurements.

DATA ACQUISITION

The left wings were mounted in Euparal on microscope slides. A total of 786 individuals were analysed (Table 2). A photograph of the ventral side of the wing was captured using a 720×576 pixels digital camera connected to a binocular microscope. A set of 14 landmarks per wing was digitized (Fig. 1), with a theoretical precision of $8.8 \mu\text{m}$, using the tpsDig2 software (Rohlf, 2009).

Wing size must be analysed in conjunction with body size to test hypotheses on flight ability. Therefore, maximal thorax length was measured in all wild individuals for comparison of wing loads (calculated here as the ratio of wing centroid size to thorax length in mm, i.e. the inverse of wing load; Yeap *et al.*, 2013). Dry mass after storage in 70% alcohol was also measured ($d = 10 \mu\text{g}$) in the subset of individuals trapped in 1998 in the Kerguelen Islands. This measure cannot be taken as a regular measure of dry mass because several compounds are solubilized in ethanol, but both measurements are highly correlated (Den Nijs *et al.*, 1996; Knapp, 2012), and the present measure was used as a relative comparison among individuals rather than an absolute assessment. These mass data allowed checking, in a linear regression, the relevance of thorax length as a surrogate of body mass in wild individuals (Fig. S1).

MORPHOMETRICS AND STATISTICAL TREATMENT

The landmark configurations were superimposed using a Generalized Procrustes Analysis (Rohlf & Slice, 1990; Dryden & Mardia, 1998; Claude, 2008). Because the Procrustes superimposition constrains the data (4 d.f. are lost, resulting in a reduction of dimensionality), Principal Component Analysis (PCA) was applied to the resulting Procrustes coordinates, and the scores on the non-null Principal Components (PC) were used as shape variables in all subsequent analyses (Debat, Debelle & Dworkin,

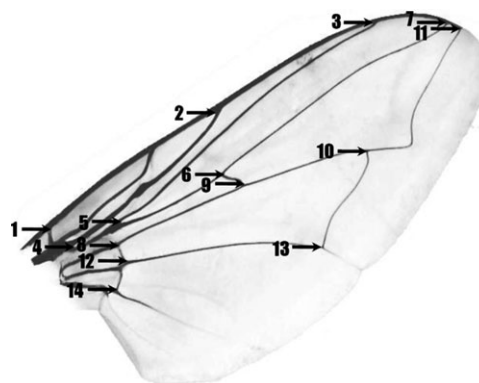


Figure 1. Position of the 14 landmarks digitized on the left wing of *Calliphora vicina* (ventral face).

2009). We used the centroid size as a measure of wing size (Claude, 2008).

MEASUREMENT ERROR

Image acquisition and landmark digitization are the two main sources of measurement error in geometric morphometrics (Arnqvist & Martensson, 1998). To assess their impact, we applied a design adapted from (Alibert *et al.*, 2001) to a subsample of 29 wings. Each wing was photographed, digitized, and, in an independent session 45 days later, it was repositioned, rephotographed, and redigitized by the same operator. This method combines the effects of image acquisition and landmark digitization, therefore allowing quantification of the overall measurement error relative to the biological variation among individuals. The statistical significance of measurement error was tested using ‘individual wing’ as the main effect in a Procrustes analysis of variance (ANOVA) (Mardia, Kent & Bibby, 1979; Klingenberg, Barluenga & Meyer, 2002).

VARIATION IN WING LOAD

We investigated whether wing load changed over time in wild individuals (hereafter referred to as

Table 2. Sample sizes of the groups analyzed

Population	Gender	8 °C	12 °C	16 °C	20 °C	Past	Recent
Kerguelen	Males	40	45	28	40	60	26
	Females	40	44	30	40	59	56
Brittany	Males	20	20	20	20	30	20
	Females	20	20	20	20	29	39

Individuals reared at the four controlled temperatures are the first offspring of the individuals trapped in the field in 2009 (‘Recent’ column). ‘Past’ samples correspond to 1998 for Kerguelen and to 1983 for Brittany.

‘allochronic change’). This was tested separately for males and females using two ANOVAs with ‘allochronic change’ (past or recent), ‘population’, and their interaction as factors. Pairwise comparisons were tested in both cases using a Tukey post-hoc procedure.

VARIATION IN WING SHAPE

Allochronic change in wing shape was tested in both genders using multivariate analysis of variance (MANOVA) (trapping year \times population). Pairwise comparisons between past and recent samples were performed for both sexes using four separate Hotelling t^2 tests. In each case, we used a Linear Discriminant Analysis (LDA) to visualize the shape change from past reference to 2009 (i.e. two groups resulting in one discriminant axis accounting for 100% of inertia). The Procrustes distance between the past and recent groups was calculated each time. As previous MANOVAs resulted in interpopulation differences in female allochronic change only, we further focused on this gender. All wild females from both populations were compared in a discriminant analysis to examine the nature of these changes.

Thermal phenotypic plasticity was investigated in both genders using MANOVAs (temperature \times population) and was further investigated in a discriminant analysis using PC scores.

COMPARING PHENOTYPIC PLASTICITY AND ALLOCHRONIC CHANGE

Allochronic and thermal shape changes were compared between the populations using Principal Coordinates Analysis (PCO, also known as metric multidimensional scaling; Mardia *et al.*, 1979; Debat *et al.*, 2006, 2009; Mitteroecker & Bookstein, 2009). This analysis is related to PCA, but allows the similarity among a set of matrices to be displayed. Because phenotypic shape changes were non-linear across the thermal range, no single shape axis could be plotted against temperature. We thus estimated the shape change for each pair of consecutive temperatures separately (i.e. for 8–12 °C, 12–16 °C, and 16–20 °C), resulting in vectors of shape changes instead of matrices. For each population, we computed the covariance vectors that depict the different components of shape variation: three vectors per population for temperature, and one vector per population for allochronic change. Thus, we obtained eight vectors of shape changes that each corresponded to a single PC (accounting for 100% of the differences in each pair), which were compared in the PCO. We used $1-r^2$ as a metric distance to compare the vectors in the PCO (Debat *et al.*, 2006, 2008, 2009).

The Procrustes ANOVA was performed using *MorphoJ* 1.02c (Klingenberg, 2011). All other statistical procedures were conducted with *R* 2.7.2 statistical software (R Development Core Team, 2008) using a prerelease of the *Rmorph* library (M. Baylac, pers. comm.) and the code provided in Claude (2008).

ETHICAL STATEMENT

Following the advice of its Scientific Council, the French Polar Institute [Institut Polaire Français Paul-Émile Victor (IPEV)] is the authority that supported this research. The sites visited did not require any access authorization. All research and data reported here were obtained in compliance with all current French laws: no permission was required for collecting these insects in the French sub-Antarctic islands and in Brittany.

RESULTS

MEASUREMENT ERROR

The measurement error was found to be negligible relative to the biological variation among individuals. The impact of relative measurement error on centroid size and shape was indeed extremely low, namely 1095.6 and 41.6 times lower, respectively, than the variation resulting from biological interindividual variation (Table 3). We consequently assume that the results are not significantly biased by measurement inaccuracies.

WING LOAD IN WILD INDIVIDUALS

We computed the wing/thorax ratio instead of the wing load and therefore higher values indicate lower wing loads (i.e. comparatively large wings). The mean wing load was lower in females than in males and in flies from the Kerguelen Islands compared with those from Brittany (Fig. 2). Therefore, males and females were further analysed separately (Table 4).

Wing load changed significantly in both genders within the time frame of our analysis, but only females’ wing load changed differentially between populations (significant interaction term, Table 4, Fig. 2). No change was detected in females from the Kerguelen Islands, whereas the wing load in females from Brittany showed a slight increase over time (note that the time period over which this was studied was longer compared with that for females from the Kerguelen Islands). Tukey’s test revealed that the significant allochronic change found in males (Table 4) resulted from irrelevant pairwise comparisons (i.e. past males from the Kerguelen Islands vs. recent males from Brittany; data not shown).

Table 3. Results from the Procrustes analysis of variance (ANOVA) for individuals photographed and digitized twice for assessing measurement error

Data set	Effect	SS	MS	d.f.	<i>F</i>	<i>P</i>
Centroid size ($N = 29 \times 2 = 68$ digitizations)	Individual	83.8	3.0	28	1095.6	***
	Error	7.9×10^{-2}	2.7×10^{-3}			
Shape ($N = 29 \times 2 = 68$ digitizations)	Individual	1.5×10^{-2}	2.2×10^{-5}	672	41.6	***
	Error	3.7×10^{-4}	5.2×10^{-7}	696		

The *F* statistic is the ratio between the variation resulting from interindividual differences and the variation induced by measurement error. All numerical data are rounded to one decimal point.

d.f., degrees of freedom; MS, mean square; SS, sum of squares.

*** $P < 0.0001$.

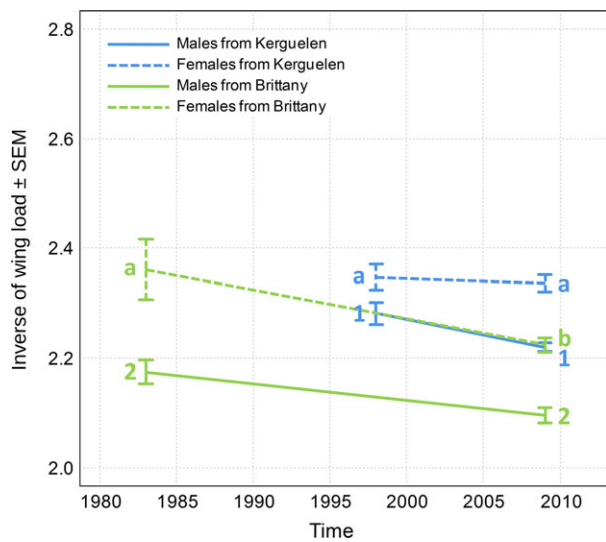


Figure 2. Inverse of wing load (wing centroid/thorax length ratio) in each group of wild individuals (expressed as mean ± SEM). Blue lines, Kerguelen Islands; green lines, Brittany; solid lines, males; dashed lines, females. Results of post-hoc Tukey tests performed for each ANOVA (one in each gender) are detailed: different numbers denote significant differences in males; different letters denote significant differences in females.

ALLOCHRONIC CHANGE IN WING SHAPE

Significant allochronic change in wing shape was detected for both genders (Table 5). The wing shape changed more in females from the Kerguelen Islands than in females from Brittany, despite a non-significant change in wing size and a shorter study time period for flies from the Kerguelen Islands. Conversely, there was no population-related difference in wing shape changes in males, as shown by the non-significant interaction term (Table 5).

The allochronic shape changes within each population and gender were further investigated using pairwise discriminant analyses, each separating past and recent samples along a single discriminant axis (Fig. 3). These results confirmed stronger changes in females from the Kerguelen Islands, with landmark shifts mostly found in the posterior region of the wing.

The statistical significance of the allochronic change illustrated in Figure 5 was checked using pairwise Hotelling t^2 tests, which demonstrated the higher magnitude of changes in females from the Kerguelen Islands, followed by males from the same population (see Procrustes distances, *F* statistics, and significance levels; Table 6). Of additional note, the time lag between the sampling sessions was shorter in this population than in that from Brittany

Table 4. Results from the two ANOVAs computed on wing loading for wild males and wild females

Data set	Effect	$F_{d.f.}$	SS	MS	<i>P</i>
Wild males ($N = 136$)	Allochronic change	13.2 ₁	0.2	0.2	***
	Population	27.3 ₁	0.4	0.4	***
	Allochronic change:population	0.1 ₁	0.0	0.0	NS
Wild females ($N = 183$)	Allochronic change	6.0 ₁	0.2	0.2	*
	Population	4.3 ₁	0.1	0.1	*
	Allochronic change:population	5.8 ₁	0.2	0.2	*

All numerical data are rounded to one decimal point.

$F_{d.f.}$, *F*-statistic/degrees of freedom; MS, mean square; NS, not significant; SS, sum of squares.

* $P < 0.05$; *** $P < 0.001$.

Table 5. Results from the two multivariate analyses of variance (MANOVAs) computed on the Principal Components (PC) scores for wild males and wild females

Data set	Effect	$F_{d.f.}$	Pillai	P
Wild males ($N = 136$)	Allochronic change	4.4 ₂₄	4.9	***
	Population	8.9 ₂₄	6.6	***
	Allochronic change:Population	1.6 ₂₄	2.6	NS
Wild females ($N = 183$)	Allochronic change	5.6 ₂₄	4.6	***
	Population	11.7 ₂₄	6.4	***
	Allochronic change:Population	2.9 ₂₄	3.1	***

All numerical data are rounded to one decimal point. Pillai trace is multiplied by 10.

$F_{d.f.}$, F -statistic_{degrees of freedom}; NS, not significant.

*** $P < 0.001$.

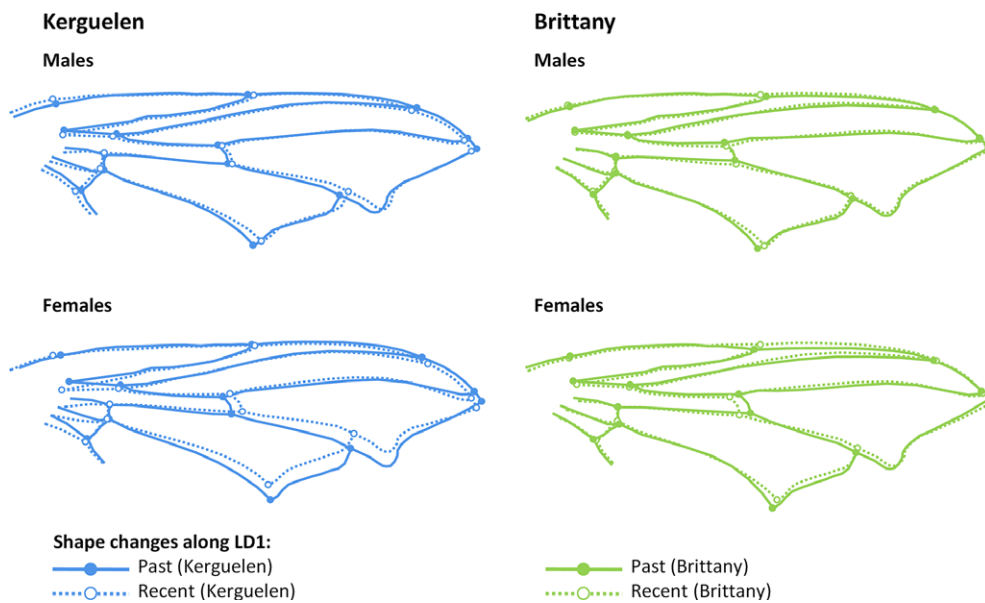


Figure 3. Allochronic shape change within each population and gender. The changes between past samples and recent samples were reconstructed in each case using separate discriminant analyses, and were amplified and correspond to a 15-unit change along the first axis (LDA1) (accounting for 100% of the inertia).

(11 years vs. 26 years), and the number of generations per year was limited to only one to two because of harsher seasons.

The stronger population dimorphism between female allochronic changes was further investigated using an LDA (Fig. 4), which significantly separated the four population-period groups (LDA1, Wilks' $\Lambda = 0.143$, $P < 0.001$; LDA2, Wilks' $\Lambda = 0.418$, $P < 0.001$). The first axis, which accounted for 62.2% of the total inertia, mainly corresponded to a population axis (Fig. 4A) and the second axis accounted for 30.4% of the total inertia and corresponded to a time axis. Populations mainly differed in the leading edge and the posterior region of the wing (LDA1). The second axis revealed a striking pattern of allochronic change in the posterior-distal half of the wing of

females from the Kerguelen Islands (Fig. 4A; bimodal density of individual projections along LDA2, Fig. 4B). By contrast, only minor variation was found in the wing of females from Brittany (unimodal density of individual projections along LDA2, Fig. 4B). Interestingly, the dispersion of points in the plane was not higher in recent samples from the Kerguelen Islands compared with past samples, indicating that variance in wing shape has not increased over time.

THERMAL PHENOTYPIC PLASTICITY OF WING SHAPE

The influence of developmental temperature on wing shape was investigated among populations and for later comparison with allochronic change. The two populations (Brittany and the Kerguelen Islands) showed

Table 6. Results from the four Hotelling t^2 tests computed on the Principal Components (PC) scores to test the allochronic effect in wild individuals

Data set	Effect	$F_{d.f.}$	Hotelling	Procrustes distance	P
Wild males, Kerguelen ($N = 86$)	Allochronic	3.8 ₂₄	1.5	6.7	***
Wild males, Brittany ($N = 50$)	Allochronic	3.5 ₂₄	3.4	5.8	**
Wild females, Kerguelen ($N = 115$)	Allochronic	6.8 ₂₄	1.8	10.4	***
Wild females, Brittany ($N = 68$)	Allochronic	2.3 ₂₄	1.3	5.9	**

All numerical data are rounded to one decimal point. Procrustes distance is multiplied by 10^3 .

$F_{d.f.}$, F -statistic_{degrees of freedom}.

** $P < 0.01$; *** $P < 0.001$.

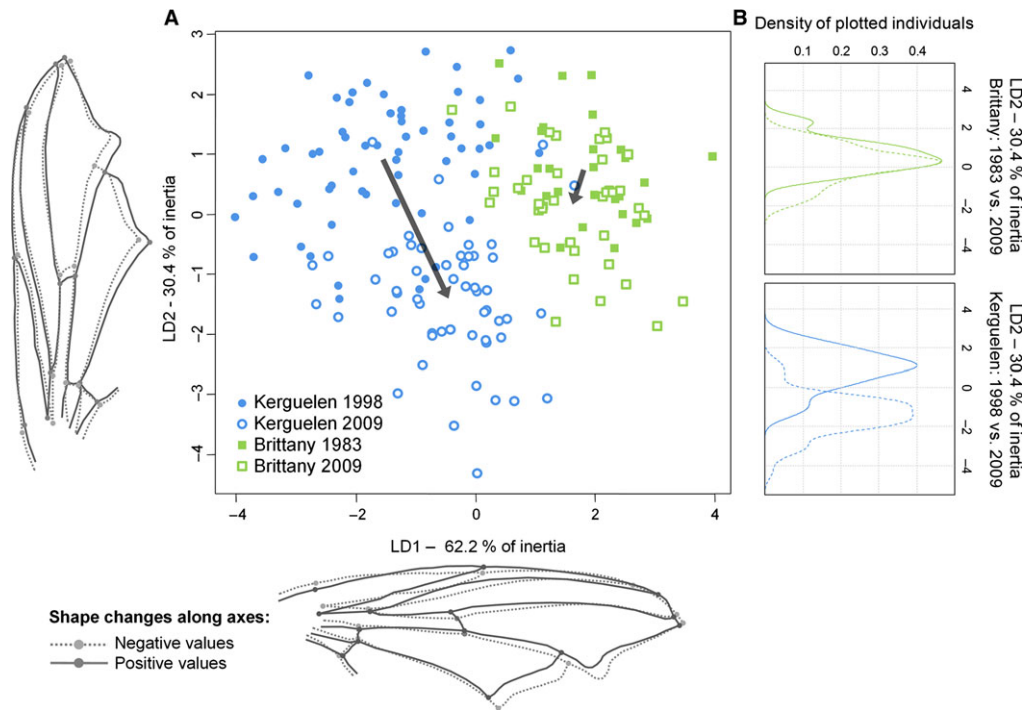


Figure 4. A, differential allochronic shape change between females of each population. Linear Discriminant Analysis (LDA): projection of the 183 wild females grouped according to trapping year and population on the first discriminant plane (LDA1, LDA2). The shape changes are amplified and correspond to a 15 unit change along each axis. Dots, Kerguelen Islands; squares, Brittany; solid symbols, past samples (1998 and 1983, respectively); open symbols, recent samples (2009). The allochronic change within each population is illustrated by the black arrow linking the centroids. The amount of inertia represented by each axis is shown in the axis legend. B, unimodal (Brittany) vs. bimodal (Kerguelen Islands) density plots of the same individuals along LDA2. Blue, Kerguelen Islands; green, Brittany; solid lines, past individuals; dashed lines, recent individuals.

different reaction norms, as illustrated by the significant interaction terms in males and females (Table 7). A discriminant analysis (Fig. 5) showed that both populations shared a common general pattern, but differed for some thermal vectors, particularly those of 8–12 °C and 16–20 °C (females only). Aside from the interpopulation comparison, the analysis revealed a strong sexual dimorphism, with the genders being separated regardless of population and developmental temperature

(LDA1, Wilks' $\Lambda = 0.001$, $P < 0.001$; LDA2, Wilks' $\Lambda = 0.008$, $P < 0.001$).

SIMILARITY BETWEEN ALLOCHRONIC SHAPE CHANGES AND WING THERMAL PLASTICITY

The angles between vectors depicting each thermal change (8–12 °C, 12–16 °C, and 16–20 °C) and allochronic change (past to recent) were computed for

Table 7. Results from the two multivariate analyses of variance (MANOVAs) computed on the Principal Components (PC) scores for males and females reared in the laboratory

Data set	Effect	$F_{d.f.}$	Pillai	P
Laboratory-reared males ($N = 233$)	Temperature	12.4 ₇₂	1.8	***
	Population	17.1 ₂₄	0.7	***
	Temperature: Population	6.3 ₇₂	1.3	***
Laboratory-reared females ($N = 234$)	Temperature	8.7 ₇₂	1.5	***
	Population	18.1 ₂₄	0.7	***
	Temperature: Population	5.1 ₇₂	1.1	***

All numerical data are rounded to one decimal point.

$F_{d.f.}$, F -statistic_{degrees of freedom}.

*** $P < 0.001$.

males and females from the two populations. Ordination in a PCO (Fig. 6) confirmed the contrasting thermal reaction norms produced by the two populations (from Brittany and the Kerguelen Islands), despite a cluster between vectors K16–20M, K16–20F, and B12–16F. The PCO also highlighted a tight clustering between equivalent thermal changes of males and females from the Kerguelen Islands, as opposed to individuals from Brittany. This result indicates less sexually dimorphic thermal reaction norms in flies from the Kerguelen Islands compared with flies from Brittany.

In the Kerguelen Islands, the allochronic changes in females and males matched the changes observed in the cold part of the reaction norms (i.e. from 8 to 12 °C, and, to a lesser extent, from 12 to 16 °C), although females tended to differentiate along the second axis (Fig. 6). No cluster appeared for changes observed in flies from Brittany. The cosines of angles among vectors (Table S1) provide quantitative information on the directions of shape changes ordinated in the PCO. In the Kerguelen Islands, these values corroborated: (1) the strong similarity between male and female thermal reaction norms (Table S1-A); and (2) the similarity between plastic changes incurred at the lowest rearing temperatures and allochronic changes observed in the wild (Table S1-A). Conversely, such associations were weak in flies from Brittany (the vectors were in nearly opposite directions at the highest thermal shift, Table S1-B), strengthening the idea of contrasting reaction norms between populations (Table S1-C).

DISCUSSION

Our results support our initial hypothesis that *C. vicina* wing incurred rapid changes during the

invasion process in the Kerguelen Islands. The different shape of the leading edge of the wing between populations may reflect different selective pressures on flight ability, this region of the wing being related to functional constraints for the flight (Dickinson *et al.*, 1999; Gilchrist *et al.*, 2000). It is not known, however, whether individuals of the strain from the Kerguelen Islands experienced notable changes in this particular region of the wing before the period considered in our study. Owing to the unknown origin of the invasive population in the Kerguelen Islands, we lack a benchmark to tease apart local adaptation to the peculiar sub-Antarctic conditions from a putative founder effect. However, the contrasting thermal reaction norms observed between populations are supportive of local adaptations (Debat *et al.*, 2008; Loh *et al.*, 2008).

SEXUAL DIMORPHISM PROBABLY REFLECTS DEVELOPMENTAL CONSTRAINTS

A relatively steady sexual dimorphism, such as that found in the present study for both wing size and shape, has been suggested to reflect morphogenesis and developmental constraints (Gilchrist *et al.*, 2000). In *Drosophila melanogaster* (Diptera, Drosophilidae), stable sexual dimorphism in wing shape was found among very distant populations and in spite of a morphological cline in wing size and shape (Gilchrist *et al.*, 2000). As reviewed in Fairbairn (1997), the smaller wings of males reflect most descriptions of sexual size dimorphism in insects, despite exceptions existing with respect to taxa, life strategies, and sexual selection. Sexual dimorphism often results from different selection regimes and energy expenditures between genders, with some heavy trade-offs primarily occurring in females as a result of egg production and dispersal (i.e. locating sexual partners and food for offspring) (Williams & Richardson, 1983; Davies, 2006). Examining this scenario in *C. vicina* will require further fitness studies.

MORPHOLOGICAL DIFFERENCES MAY BE RELATED TO DIFFERENT ENVIRONMENTAL PRESSURES

It is unclear whether the morphological variations recorded in our study are adaptive. Indeed, the investigation of phenotypic variation among wild individuals allows a broad range of environmental parameters to be investigated (Hendry & Kinnison, 1999), but makes it difficult to tease apart adaptive and non-adaptive features. However, there is substantial support in the literature for a strong relationship between wing morphologies and local adaptations. Wing size and shape can be under distinct selective pressures (Hoffmann & Shirriffs,

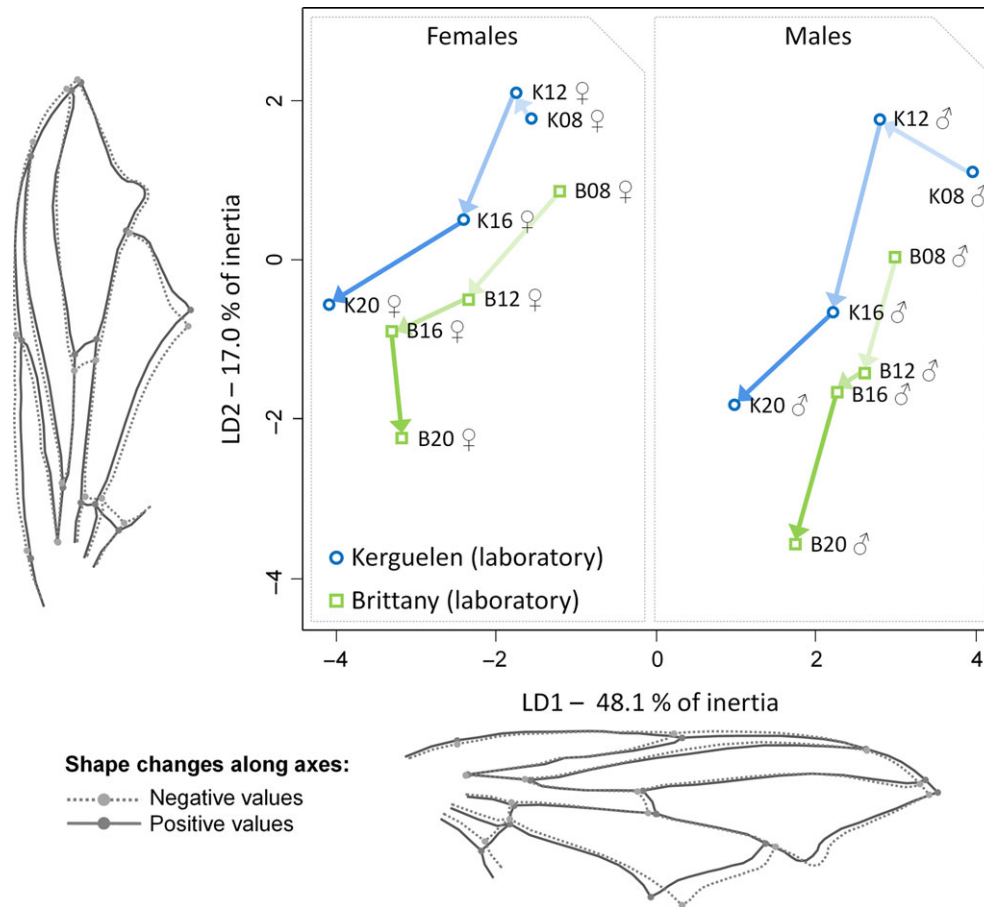


Figure 5. Thermal plasticity of wing shape among genders and populations. Linear Discriminant Analysis (LDA): projection of the 467 individuals reared in the laboratory on the first discriminant plane (LDA1, LDA2). For ease of interpretation, only centroids were plotted. The shape changes are amplified and correspond to a 15-unit change along each axis. Dots, Kerguelen Islands; squares, Brittany. The thermal reaction norms within each population are illustrated by the arrows linking the temperature centroids. The amount of inertia represented by each axis is shown in the axis legend.

2002): although size may be subject to directional selection, shape is likely to have been subject to optimizing selection and canalization, resulting, for example, in the conserved wing structure of all Drosophilidae species (Gilchrist & Partridge, 2001; Mezey & Houle, 2005). It was further reported that 'evolutionary changes in flight-morphology are symptomatic of populations with altered costs and benefits of dispersal' (Van Dyck & Matthysen, 1999). Evidence of distinct shapes within a species consequently reflects the coexistence of multiple levels of optimality that are dependent on local conditions (Weber, 1990; Gilchrist *et al.*, 2000). Such geographical variations in wing shape have been frequently found among distinct populations of *D. melanogaster* (Gilchrist *et al.*, 2000; Gilchrist & Partridge, 2001; Hoffmann & Shirriffs, 2002). They are common in cosmopolitan species (Mayr, 1963) and often arise as a result of local adaptations to

biotic and abiotic factors in particular areas (Ricklefs & Miles, 1994; Hoffmann, Shirriffs & Scott, 2005). Thus, and as hypothesized in *Drosophila lummei* (Haas & Tolley, 1998), wing shape variations observed between populations in the present study may reflect differences in environmental pressures on wing characteristics.

Information remains limited about shape patterns, aerodynamics, and their associated adaptive significance for flight (Gilchrist *et al.*, 2000). However, the anterior-proximal region of the wing may be directly impacted by the functional constraints for flight dispersal, as it contributes towards maintaining the axis of rotation near to the leading edge during flight (Dickinson *et al.*, 1999; Gilchrist *et al.*, 2000). Noticeable differences in the anterior-proximal region of the wing were found between females from the two populations of *C. vicina*, thereby supporting our second hypothesis that pressures on flight morphologies

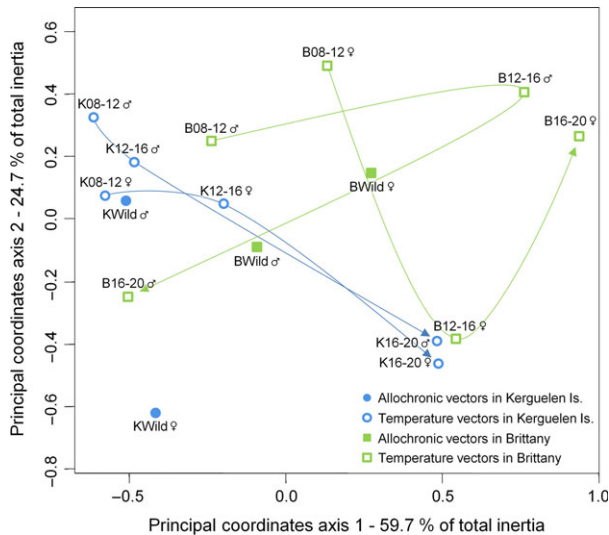


Figure 6. Comparison of shape vectors resulting from thermal shifts between consecutive temperatures and allochronic changes. Principal Coordinates Analysis: projection on the first plane of eight shape vectors depicts either the changes induced by temperature shifts in the laboratory or the allochronic changes. Each symbol represents a single matrix. Dots, matrices for Kerguelen Islands; squares, matrices for Brittany; solid symbols, allochronic changes (1983–2009 and 1998–2009); open symbols, temperature shifts. The trajectories through the whole thermal range for each gender of each population are illustrated by the arrows. The amount of inertia represented by each axis is shown in the axis legend.

may differ between the two geographical areas. We only found limited allochronic change in the anterior-proximal region of the wing, but it is usually less variable than the posterior-distal region (see results in Pezzoli *et al.*, 1997; Gilchrist *et al.*, 2000), arguably because of the aforementioned functional constraints. Additional studies will be necessary to test whether the differences in the leading edge between the two populations resulted from the introduction of *C. vicina* in the Kerguelen Islands before our first sample or pre-existed in the population of origin.

From an ecological perspective, novel selection pressures – either stronger or relaxed – on the wing of *C. vicina* from the invaded area would be relevant. As emphasized in (Medeiros & Gillespie, 2011), insect flightlessness is more likely to appear in insular, windswept, and alpine environments, such as the Kerguelen Islands (Le Roux *et al.*, 2002). In addition, the main trophic resources are present in vast quantities at coastal areas (Vernon, 1981; Lebouvier *et al.*, 2011), thereby reducing the need for long-range movements, as illustrated in most native saprophagous insects that evolved flightlessness partly in response to food distribution

(Vernon, 1981; Chevrier, 1996). However, it has been reported that transient habitats – such as carrion for saprophagous species – require colonist individuals to have a high dispersal power (Hanski, 1987). Despite the most abundant amounts of food being present along the coastlines in the Kerguelen Islands, where individuals were sampled, sustained ability for long-range dispersal in these islands may be beneficial for reaching distant and geographically isolated carrions, thereby providing an advantage over native competitors. This putative advantage is of paramount importance because *C. vicina*, in the Kerguelen Islands' summer environment, is mainly attracted to carrion during the first 3 days of decomposition (Chevrier *et al.*, 1997). The lower wing loads found in individuals from the Kerguelen Islands compared with those from Brittany support this idea, as the higher energy expenditures in wing development imply altered costs and benefits of flight. The absence of increased variance in the leading edge of wings of individuals from the Kerguelen Islands supports the idea of non-relaxed selection on flight, and it may represent a competitive advantage for *C. vicina* in the invaded islands.

Finally, our trapping design is common for flies, but is targeted towards capturing active flyers. Owing to this potential sampling bias, we cannot exclude the possibility that the genotypes of the least efficient flyers were missed with this experimental design. If so, it is noteworthy that these individuals are more likely to ensure an adequate fitness in the Kerguelen Islands compared with Brittany, given the mode of distribution of food and mating habitats detailed above. Meanwhile, alone, this possible bias could not explain the quicker and larger allochronic change found in the Kerguelen Islands.

IS THE FAST ALLOCHRONIC CHANGE IN FEMALES FROM KERGUELEN ADAPTIVE?

The allochronic change in wing shape was largest in females from the Kerguelen Islands, even though the sampling interval was shorter (by half) than that for females from Brittany. The posterior components of the wing that changed over time are, to our knowledge, not fully understood as functional constraints for flight (Dickinson *et al.*, 1999; Gilchrist *et al.*, 2000). They may be associated with other life-traits or may be non-adaptive.

Many natural pressures may primarily apply to females as a result of trade-offs in energy allocation to reproduction vs. dispersal (Williams & Richardson, 1983; Van Dyck & Matthysen, 1999; Davies, 2006). *Calliphora vicina* is known as a good disperser: energetic allocation of females is biased towards flight

compared with other saprophagous Calliphoridae, such as *Lucilia sericata*, but this comes with a cost in egg production, with comparatively lower fecundity (Williams & Richardson, 1983; Davies, 2006). Such trade-offs are prime components of invasive success (Rosecchi, Thomas & Crivelli, 2001; Chown *et al.*, 2007), and their morphological covariates are therefore of particular interest (Laparie *et al.*, 2010). Sex-specificity of the covariation between wing shape and dispersal activity was reported in the butterfly *Melitaea cinxia* (Lepidoptera, Nymphalidae) and included the posterior region of the hindwing (Breuker, Brakefield & Gibbs, 2007) (see also Van Dyck & Matthysen, 1999). These authors argued that this sex-specificity was most probably the result of wing shape being associated with different life-traits depending on gender, resulting in different selection pressures on wing shape. Further studies should now address whether the comparatively quick change in wing shape of females from the Kerguelen Islands reflects changes in dispersal vs. reproduction strategies as a result of the particular resource availability in the Kerguelen Islands (Vernon, 1981; Lebouvier *et al.*, 2011).

Alternatively, we cannot exclude a founder effect to contribute explaining the larger and faster change found in individuals from the Kerguelen Islands. As emphasized in Sax *et al.* (2007), the reduced genetic variance resulting from bottlenecks may increase the relative significance of genetic drift, or gene flows in the case of multiple introductions, on phenotypes. Nevertheless, because of their extreme geographical remoteness, low human visitation, and stringent biosecurity measures, incoming gene flows are largely precluded in the Kerguelen Islands (Frenot *et al.*, 2005; Lebouvier *et al.*, 2011).

THERMAL PHENOTYPIC PLASTICITY MAY HELP IN UNDERSTANDING ALLOCHRONIC CHANGES

Wing shape plasticity to temperature is common in insects (see, for example, work on the *Drosophila* genus in Imasheva *et al.*, 2000; Debat *et al.*, 2003, 2008), yet remains difficult to correlate with function (Gilchrist *et al.*, 2000; Debat *et al.*, 2008). The two populations we studied produced contrasting thermal reaction norms, especially at low temperatures. Although the functional outcomes of the associated morphological differences remain to be determined, the clear difference detected at low temperatures might tentatively be related to a local adaptation to the thermal conditions encountered in the Kerguelen Islands. Reaction norms are indeed ‘mixtures of adaptations and constraints’ (Stearns, 1989), and altered plasticity is considered as one of the primary adaptive responses to novel environmental conditions.

The above view remains highly hypothetical though, and thus our results cannot firmly answer our third hypothesis, or exclude non-adaptive differences or direct effects of developmental constraints. However, this hypothesis is supported by the combination of PCO ordination and cosines of angles between vectors. It demonstrated that, in the Kerguelen Islands only, the vectors depicting actual allochronic change in nature clustered with the shape vectors that corresponded to cold temperature shifts. As shown for *Drosophila* wings (Debat *et al.*, 2009), temperature may alter the direction of available phenotypic variation, in addition to changing selective pressures. Considering plasticity as a mechanism promoting different evolutionary solutions is controversial and has long been debated (see for instance De Jong, 2005). Yet, several studies have discussed how phenotypic plasticity – as a primary parameter or in parallel with genetic variation – can alter the potential for evolution (West-Eberhard, 2003; Palmer, 2004; Badyaev, 2005; Braendle & Flatt, 2006; Debat *et al.*, 2009). Schematically, an altered phenotype expressed in a new environment as a result of phenotypic plasticity will partly mitigate the novel selection pressures in the short term, meaning that, in turn, it will alter their influence and the future adaptive responses. Therefore, the product of phenotypic plasticity may not only be a consequence of constraints, but may also feed back individuals’ sensitivity to these constraints and ultimately foster later evolution.

PERSPECTIVES

We combined examinations of allochronic and plastic changes of the wing in an active flyer currently invading an ecosystem where flightlessness has always been the rule. This biological system provides unique opportunities for tackling ecologically driven rapid evolution and delineating ecomorphology of flight. As a first step towards achieving these challenging tasks, we documented the morphological changes that actually occurred within this system, and discussed their potential outcomes. Complementary data are now necessary to address their evolutionary significance and putative genetic architecture. Additional studies will be required to track the consistency of the ongoing allochronic kinetics over the long term, and to draw directional predictions for evolution in the sub-Antarctic conditions.

Evolutionary responses of alien species to their newly colonized environments can alter their spread and their interactions with the native biota, thus stressing the relevance of such studies in evolutionary ecology and conservation (Huey *et al.*, 2005).

Additionally, global warming may interact with dispersal-related morphologies, but will also broaden seasonal activity periods, speed up development rates, and increase winter survival. Ultimately, such effects may facilitate the invasion dynamics by allowing extra generations each year and broadening habitat suitability. A better understanding of *C. vicina*'s invasion and its life-history changes through the process is therefore crucial in the Kerguelen Islands, where the native and highly endemic biota is endangered by both aliens and climate change.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Linear regression between maximal thorax length and dry mass after ethanol storage in *Calliphora vicina*. The regression is based on wild individuals of *Calliphora vicina* trapped in the Kerguelen Islands in 1998. Grey dots: females; black dots: males. 95% confidence intervals are displayed.

Table S1. Cosines of angles between the shape vectors depicting thermal changes (08–16, 12–16 and 16–20) and allochronic change (past–recent) within the Kerguelen Islands (A), within Brittany (B), and between the two populations (C).