

Research

Female butterflies modulate investment in reproduction and flight in response to monsoon-driven migrations

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Migratory species may display striking phenotypic plasticity during individual lifetimes. This may include differential investment in body parts and functions, differential resource use and allocation, and behavioural changes between migratory and non-migratory phases. While migration-related phenotypic changes are well-reported, their underlying mechanisms are usually poorly understood. Here we compare individuals from migratory (reproductive diapause) and non-migratory (reproductive) phases of closely related aposematic butterfly species to study how sexual dimorphism and migratory behaviour underlie significant morphological tradeoffs, and propose a plausible scenario to explain the migration-related phenotypic plasticity observed in females of migratory species. We found that female butterflies invested significantly more in their abdominal mass compared to males irrespective of their migratory phase, and underwent a clear shift in their body mass allocation after the switch from the reproductive diapause phase to the reproductive phase. In reproductive phase, females invested much more in reproductive tissue. This switch occurred as a result of increased abdominal mass (i.e. reproductive tissue mass) without significant reduction in the thoracic mass (i.e. flight muscle mass). Migratory males, however, were not significantly different from non-migratory males in terms of relative investment in flight and reproductive tissues. These patterns were consistent between migratory and non-migratory aposematic species within and across clades. While migratory habits may influence the physiology and behaviour of both sexes, long-distance migration affected female morphology much more markedly compared to that of males. These results show the sex-specific nature of adaptations to migratory behaviour, and suggest that seemingly disparate life-history traits such as aposematism and migration may have similar influences on the lifetime energetic investments of insects.

Introduction

Many animals often respond to unfavourable environmental changes in their habitat by migrating to other areas. Seasonally predictable, annually recurring unfavourable changes in habitat conditions have shaped the evolution of spectacular trans-continental

and latitudinal migrations in many animals (Williams 1957, Dingle 2006, Roff and Fairbairn 2007, Chapman et al. 2015). Migratory habits influence multiple life history traits related to flight versus reproductive morphology and physiology (Dixon et al. 1993, Zera and Denno 1997, Alerstam et al. 2003, Hanski et al. 2006). These traits are under selection pressures that vary spatio-temporally, i.e. during migratory and non-migratory phases in an organism's lifespan, and in the landscapes where these phases occur (Thomas et al. 1998, Hill et al. 1999, Merckx and Van Dyck 2006). In spite of the importance of these selection pressures and seasonal strategies in life histories of migratory species, there is poor understanding of how short-lived migratory organisms such as insects modulate their reproductive investment relative to the migratory phases. For instance, body size and flight morphology may have significant impacts on dispersal ability (Alerstam et al. 2003). Therefore, insects in migratory phases should invest more in flight muscles (thorax) and little in reproductive tissue (abdomen) (insect thoraxes are largely muscles, and abdomens consist mostly of reproductive tissue, developing ova, and fat bodies) (Hocking 1958, Gibo and McCurdy 1993, Hughes et al. 2003, Stjernholm et al. 2005). Do they do this by elevating investment in flight morphology, or by reducing relative investment in reproductive organs while maintaining the relative investment in flight morphology in migratory phases?

We tested this by studying relative investment in reproduction versus flight morphology of danaine milkweed butterflies (Lepidoptera: Nymphalidae: Danaini) in an annual migration across peninsular India (Fig. 1) (Kunte 2005). In migratory species, butterflies cycled through three phases: 1) migratory phase with reproductive diapause, 2) post-migratory swarming phase with reproductive diapause, and 3) reproductive phase (Fig. 1b). We extensively sampled danaine butterflies in all three phases, to test whether migrating and reproductive butterflies invested differentially in flight (thorax mass) and reproduction (abdomen mass). Migratory flight would be much more energetically demanding if butterflies have investments in abdomen mass during the migratory phase. Therefore, we hypothesized that butterflies reduce investment in abdomen mass, and increase relative investment in flight, during migratory phases. Females, which usually have a much greater investment in egg-production and overall reproductive tissue compared to males (Berwaerts et al. 2002), may theoretically modulate the relative investment in flight (thorax) versus reproduction (abdomen) in four ways (Fig. 2l): (a) Females may have, on average, similar thoracic mass to that of males but with a higher abdominal mass (hypothetical orange data points in top right corner in Fig. 2l). (b) Females may have a smaller thorax than that of males but with a heavier abdomen (hypothetical pink data points in top left corner in Fig. 2l). (c) Females may have a smaller thorax than males, but similar abdominal mass (hypothetical violet data points in bottom left corner in Fig. 2l). (d) Females may have larger thoraxes and abdomens compared to males (not illustrated in Fig. 2l, but these hypothetical data points would appear to the right of the orange data points).

It is not clear which ones of these strategies are employed by female butterflies as they cycle through migratory and post-migratory reproductive diapause phases, and post-dispersal reproductive phase.

The danaine butterflies are aposematic (i.e. chemically defended with distinctive colouration to warn predators), which have different flight/reproductive morphology compared to non-aposematic (palatable) butterflies (Srygley and Chai 1990, Marden and Chai 1991). By conducting multiple within- and across-group comparisons with other aposematic species, we tested the above hypotheses regarding the differential investment in flight-related tissue and abdomen mass in migratory and non-migratory phases. The following work shows that migratory behaviour affects the two sexes differentially, with females being much more sensitive to switches in reproductive versus migratory phases with respect to relative investment in reproduction and flight morphology.

Material and methods

Milkweed butterfly migrations in peninsular India

Peninsular India has interesting geography and climate that exert ecological selection on adaptation to wet evergreen forests as well as drier scrublands in a mosaic across the peninsula. The southwestern monsoon from the Arabian Sea is the primary source of moisture for the evergreen forests on the crest and the slopes of the Western Ghats and the western coastal strip during June to September. The northeastern monsoon, on the other hand, picks up moisture from the Bay of Bengal and causes milder showers along the eastern coast and plains of southern India between October and January. This complementary, dual monsoonal system drives the unique longitudinal migration of four species of danaine butterflies in peninsular India, as illustrated in Fig. 1 and summarized below (Kunte 2005). This migration appears to have evolved in response to a combination of the following two conditions: 1) torrential south-western monsoonal rains and associated foggy climate for extended periods in the Western Ghats and the coastal strip, when thermal conditions are not favourable to adult butterflies for most of the monsoon, and 2) the apparent lack of diapause in early stages (eggs, caterpillars and pupae) of these migratory danaine species. Because of these two factors, freshly eclosed adult butterflies migrate eastward towards the drier plains and low-lying hills of the Eastern Ghats as the pre-monsoon showers arrive. These migratory swarms settle down in spots sometimes for weeks, before the oncoming southwestern monsoon drives them further eastward. During migration and the post-migratory swarming phase, butterflies remain in reproductive diapause, and begin reproduction after a few weeks. Between early August and October, these adults which migrated eastward, mate and lay eggs, and probably all die after reproduction. There appears to be a single generation in the eastern plains, whose dynamics are not yet understood. The newly eclosed progeny in the eastern plains starts the reverse westward migration to

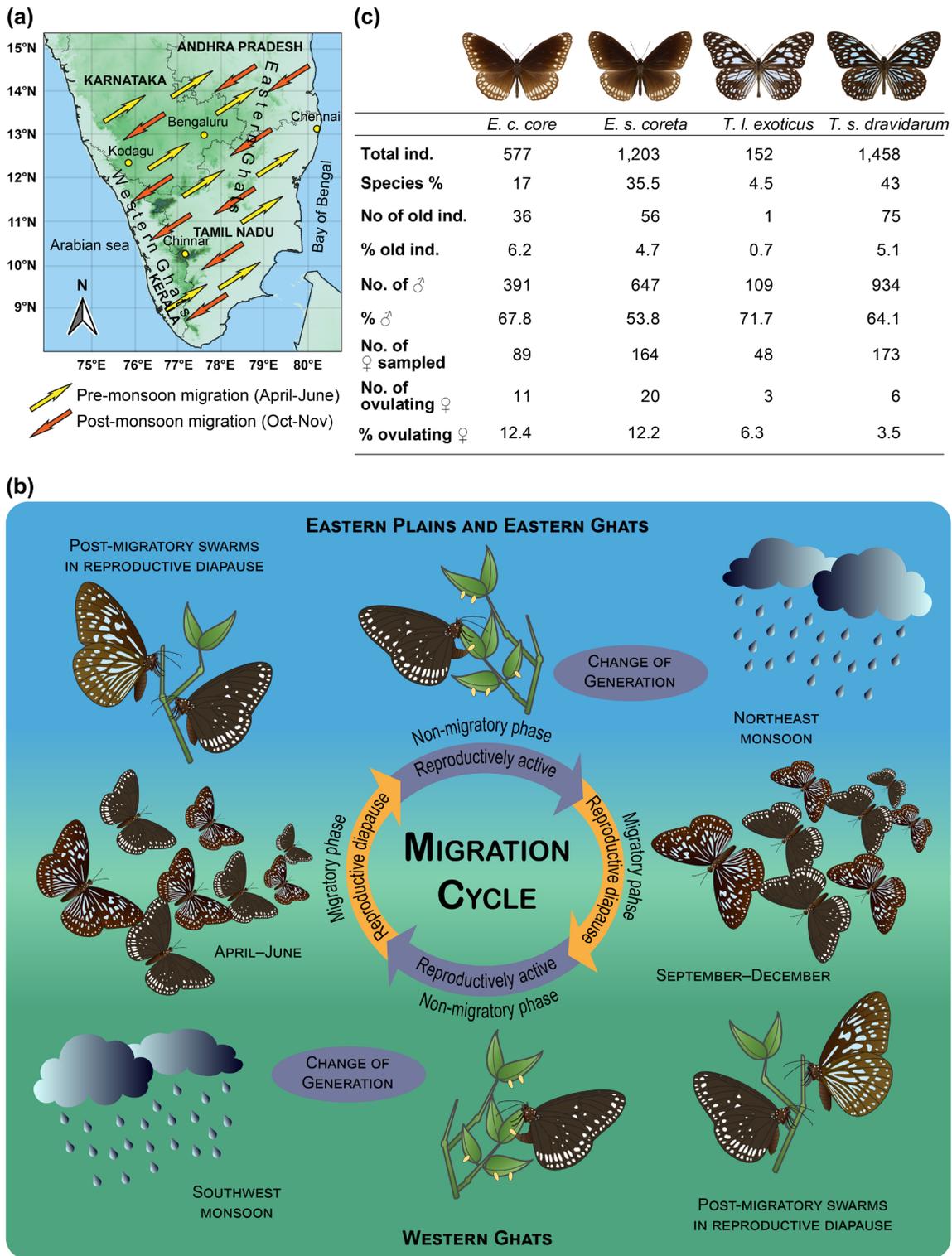


Figure 1. The longitudinal butterfly migrations in peninsular India. The danaine milkweed butterflies undertake annual migrations, in which movement patterns (a) and the alternating cycles of migration, reproductive diapause and reproductive phases are driven by the unique southwestern monsoon in India (b). Also shown is the composition of migratory swarms in terms of species, sex ratios, and reproductive status of migrating butterflies (c, and Supplementary material Appendix 1 Table A1). The four species that form the migratory swarms are: *Euploea core core*, *Euploea sylvester coreta*, *Tirumala limniace exoticus* and *Tirumala septentrionis dravidarum*. The general model of migratory routes and reproductive phases (a–b) is based on a previous study (Kunte 2005).

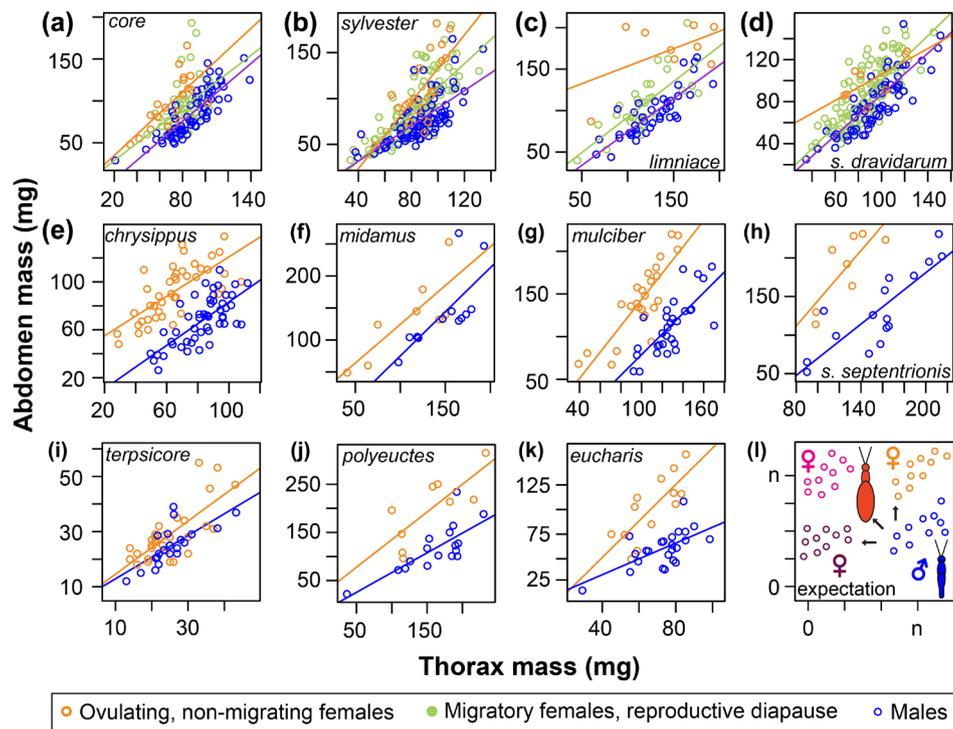


Figure 2. Sexually differential investment in flight versus reproductive tissue in the migratory danaine butterflies and other non-migratory species. Shown here are linear regressions of thoracic mass (flight morphology) and abdominal mass (reproduction). (a–d) migratory danaine species, in which females in reproductive diapause (during migration and post-migratory swarming) and ovulation (non-migratory) phases are plotted in two separate groups. (e–h) non-migratory danaine species lacking reproductive diapause. (i–k) non-migratory non-danaine species lacking reproductive diapause. (l) three ways in which female investment in flight morphology versus reproduction may theoretically differ in migratory and non-migratory phases, as compared to investment by males. These three ways may be achieved by differentially investing in thoracic versus abdominal mass. Species represented are: migratory danaines: *Euploea core core* (a), *Euploea sylvester coreta* (b), *Tirumala limniace exoticus* (c), *Tirumala septentrionis dravidarum* (d). Non-migratory danaines: *Danaus chrysippus chrysippus* (e), *Euploea midamus rogenhoferi* (f), *Euploea mulciber mulciber* (g), *Tirumala septentrionis septentrionis* (h). Non-migratory non-danaine aposematic species: *Acraea terpsicore* (i) (Nymphalidae: Heliconiinae), *Byasa p. polyeuctes* (j) (Papilionidae), and *Delias eucharis* (k) (Pieridae). Also see Table 1–2.

the Western Ghats between October and early December, possibly depending on the retreat of the monsoon and their eclosion schedules. Back in the Western Ghats, they remain in large swarms of thousands to hundreds of thousands of individuals largely resting in evergreen forests up to January, when they are in reproductive diapause, before they start dispersing and laying eggs in the forested and otherwise wooded areas of the Western Ghats and the western coastal strip. They finish this generation before the pre-monsoon showers associated with the south-western monsoon commence in April or May. This cycle of breeding, migration and brief reproductive diapause in relation to the monsoons thus ensures that the migrating swarms in this bidirectional annual migration are predominantly composed of successive generations of freshly-eclosed butterflies, as shown by additional data presented in this paper (Fig. 1c, Supplementary material Appendix 1 Table A1).

Study location and period

We observed the pre-monsoon migratory swarms of butterflies passing through various localities in Bengaluru (formerly

known as Bangalore) every year from 2012 to 2016 (Fig. 1c, Supplementary material Appendix 1 Table A1). We collected population data from the migrating swarms between March and June (2012–2016), and once between August and September (2012). We caught individuals with butterfly nets, and noted their species, sex, and wing condition (heavy wing wear = old individual, no or little wing wear = freshly eclosed individual). Although *Danaus genutia*, *Parantica aglea* and *Euploea mulciber* are known to be temperature-driven local migrants in Taiwan (Wang and Emmel 1990), they do not participate in these monsoon-driven migrations in peninsular India, and were thus not considered as migratory species in our study. The data collected from Bengaluru swarms were used to calculate most of the summary statistics in Fig. 1c. From the females collected for morphometric measurements in Bengaluru (Karnataka) and near Chittoor (Andhra Pradesh), we calculated the number and proportion of ovulating females in Fig. 1c. We also collected additional population data from Chinnar Wildlife Sanctuary (Kerala), and Kodagu (Karnataka) (Supplementary material Appendix 1 Table A1).

Butterfly collection and morphometry

We sampled females from the migratory swarms in Bengaluru and Chittoor to assess their reproductive status. We sampled non-migrating individuals of the migratory species, and all other migratory and non-migratory danaine and non-danaine aposematic species, from across southern and northeastern India throughout the year. The three non-danaine aposematic species we used to compare against danaines were those local species for which we had a large enough sample size to conduct a statistically meaningful comparison. We dissected some of the individuals collected from the migratory swarms to collect the following morphometric data and assess reproductive status within a few hours of sampling: (a) thorax mass, (b) abdomen mass, (c) whether the females were mated, as judged from the presence of spermatophores, (d) whether the female reproductive organs were developed, and (e) whether females were ovulating and carrying mature ova. We measured thorax and abdomen masses to the nearest milligram using a digital weighing balance (0.1 mg resolution), and checked reproductive status under a dissecting microscope. The thorax of butterflies is composed mainly of flight muscles with minor contribution from chitin and scales (Hocking 1958, Hughes et al. 2003). The abdominal mass consists mostly of reproductive tissue, developing ova, and fat bodies in the reproductive phase, and under-developed reproductive organs and a lot of fat bodies during migration (Gibo and McCurdy 1993), when fat acts as the energy reserves for migrating insects (Cenedella 1971, Brown and Chippendale 1974, Downer and Matthews 1976). Therefore, we used raw thoracic and abdominal masses as a measure of investment in flight and reproduction, respectively, in the regression analysis in Fig. 2 and thorax:abdomen ratios in Fig. 3.

Determination of migratory status

Migrating individuals are usually in adult reproductive diapause, characterized by arrested reproductive tract development, increased longevity, and increased abdominal fat stores (Dockx 2012). At the end of the migratory phase, the reproductive tracts develop and ova mature before breeding ensues (Herman et al. 1989). We determined the reproductive status of females as follows: (a) under-developed reproductive organs such as ovaries and colleterial glands, and the absence of mature ova and spermatophores, was taken as an indication of reproductive diapause (RD), and (b) developed reproductive organs and the presence of mature ova indicated reproductively active, ovulating (OV) females. We considered all the males in two of the migratory species and populations (*E. s. coreta* and *T. s. dravidarum*) as migratory individuals, since they were all collected from migratory swarms and it was not possible to determine their reproductive status based on dissections. In the other two species (*E. c. core* and *T. l. exoticus*), we collected males from outside the migratory range of the species in northeastern India, and we classified them as non-migratory, reproductively active

males. We did not include data from these non-migratory males in Fig. 2 and 3 in order to maintain consistency with males of the other two migratory species. However, we conducted statistical tests to ascertain whether these males significantly differed in terms of thoracic and abdominal masses from their conspecific males in migratory phases within peninsular India.

Statistical analyses

We performed all statistical analyses in R (<www.r-project.org>). We checked for normality of data using the Shapiro–Francia normality test in the package ‘*nortest*’ (Gross and Ligges 2015). Pairwise comparisons consisted of one-tailed (Table 3) and two-tailed (Supplementary material Appendix 1 Table A3) Student’s t-tests and Wilcoxon–Mann–Whitney tests for normally and non-normally distributed data, respectively. For comparisons across multigeneric groups of all aposematic butterflies (Fig. 3c), we used the Kruskal–Wallis rank sum test, followed by a Dunn’s test for nonparametric pairwise comparisons with Bonferroni correction from the package ‘*dunn.test*’ (Dinno 2015, <<https://CRAN.R-project.org/package=dunn.test>>), since data from some of the groups were non-normally distributed.

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.3tn63>> (Bhaumik and Kunte 2017).

Results

The migratory swarms of the danaine milkweed butterflies (Nymphalidae: Danainae) were composed of four species, among which *Tirumala septentrionis dravidarum* and *Euploea sylvester coreta* were predominant (Fig. 1c, Supplementary material Appendix 1 Table A1). Migrating individuals showed little wing wear, indicating that most of them (ca 95%) were freshly eclosed (Fig. 1c). The sex ratios of migrating butterflies deviated significantly from the expected 50:50, being male biased (exact binomial test, *E. c. core*: proportion of males = 0.68, $p < 0.0001$; *E. s. coreta*: proportion of males = 0.54, $p = 0.0047$; *T. l. exoticus*: proportion of males = 0.72, $p < 0.0001$; *T. s. dravidarum*: proportion of males = 0.64, $p < 0.0001$; Fig. 1c). Females sampled from migratory and post-migratory swarming phases were largely unmated and not ovulating, i.e. they were in reproductive diapause (RD females), as evidenced by the absence of spermatophores, underdeveloped reproductive organs, and fat bodies in the abdomen (Fig. 1c). This indicated that mating and reproduction perhaps ensued only as butterflies dispersed from the migratory swarms and entered the ovulating phase (OV females).

To find out how males and females differed in their relative investments in flight and reproduction, we plotted thoracic and abdominal masses of males and females of a range

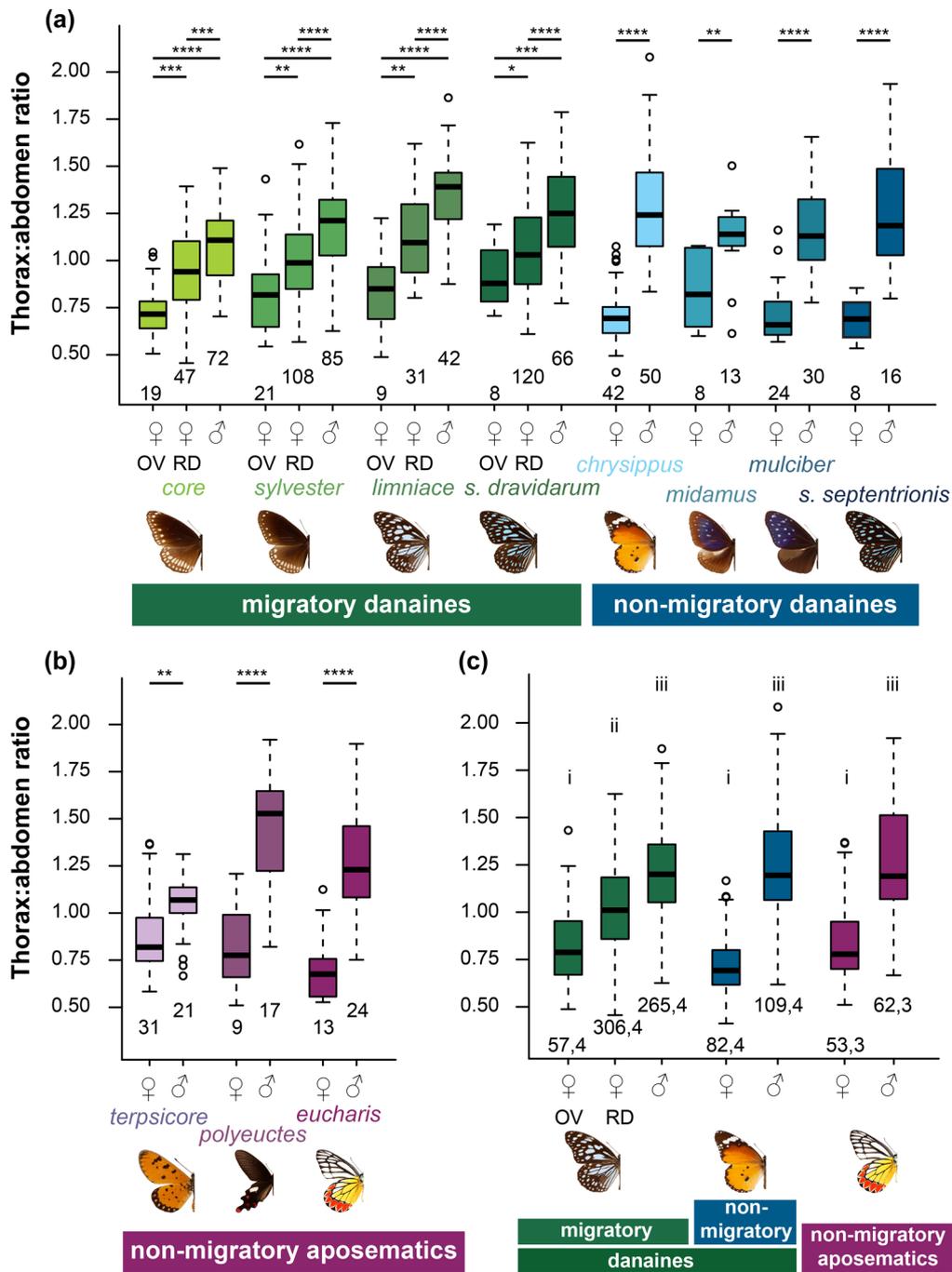


Figure 3. Overall patterns of differential investment in flight versus reproductive tissue among the sexes and migratory phases of aposematic butterflies. Differential investment is calculated as the ratio between thoracic mass (investment in flight muscles) and abdominal mass (investment in reproduction). Panel (a) compares migratory and non-migratory species (or populations in case of *Tirumala septentrionis*) of danaine butterflies. OV = ovulating, non-migratory females during the reproductive phase, and RD = females in reproductive diapause during the migratory phase. Males associated only with migratory phase were included in this analysis. Panel (b) compares investment in reproduction versus flight in non-danaine aposematic butterfly species from three different families. Panel (c) shows thorax:abdomen ratios of all males and all females across all the aposematic species pooled together. Numbers below boxplots (panel a–b) indicate sample sizes and those below boxplots (panel c) indicate the total number of individuals of the specified number of species. Asterisks denote significant statistical differences (p-values): * ≤ 0.05 , ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. In panel (c), roman numerals indicate groups of values that are statistically different from each other. Species represented are the same as that in Fig. 2 (also see Table 3).

of migratory and non-migratory milkweed butterflies (Fig. 2). In the four migratory milkweed butterfly species, there were no consistent differences between either thorax or abdomen masses of males and the two phases of females, i.e. males or females did not have consistently larger or smaller thoraxes and abdomens (Fig. 2, Supplementary material Appendix 1 Table A2, A3). In cases where males and females of a species had thoraxes of different sizes, females always had smaller thoraxes, indicating that females did not modulate their investments in flight following the theoretical expectation '(d)' above (Supplementary material Appendix 1 Table A2, A3). However, females consistently had heavier abdomens compared to males, indicating that females invested more in abdominal tissue irrespective of the migratory or reproductive status (Table 1, 2, Fig. 2). Reproductive females had even larger abdomens compared to migratory females in reproductive diapause, but similar thoracic masses (Table 1, 2, Fig. 2), indicating that females modulated their relative investment in reproductive tissue and abdominal mass in different stages of migration and reproduction by differentially investing in the abdominal mass alone, and not by changing thoracic mass. There were no consistent differences between thorax masses of males and the two phases of females (Supplementary material Appendix 1 Table A3), indicating that both males and females may have similar thoracic masses irrespective of the migratory behaviour and status. However, males invested relatively little in abdomen, and females changed their flight

morphology by investing relatively little in reproductive tissue during the migratory phase, by preferentially investing in the fat body during the migratory phase, and increasing the investment in reproductive tissue in the post-migratory reproductive phase. These patterns usually supported the hypotheses in Fig. 2I-i and 2I-ii.

The relative investment in reproduction and flight may also be compared as a ratio of thorax:abdomen mass (Fig. 3). This showed consistent patterns across all migratory danaine species: 1) males had heavier thoraxes (i.e. relatively lighter abdomens) compared to females of either migratory or reproductive phase, 2) reproductive females had heavier abdomens compared to migratory females and males, and 3) migratory females had intermediate flight/reproductive morphology between reproductive females and males (Fig. 3, Table 3). For danaine species in which some populations are migratory and some are non-migratory (*E. c. core* and *T. l. exoticus* in peninsular India versus in northeastern India), males from migratory and non-migratory populations had similar thorax:abdomen ratios, i.e. males had similar flight/reproductive morphology irrespective of the migratory habit (*E. c. core* non-migratory males compared to migratory males: $t(28.2) = -0.2945$, $p = 0.7705$; *T. l. exoticus* non-migratory males compared to migratory males: $t(17.5) = 0.3072$, $p = 0.7623$). We did not have sufficient data to make a similar comparison between reproductive females in migratory and non-migratory populations of these two species.

Table 1. Regressions between thoracic (flight) and abdominal (reproduction) masses of migratory and non-migratory danaine and other aposematic species, as analysed by linear regressions on butterfly species shown in Fig. 2. OV = Ovulating females in reproductive phase, RD = females in reproductive diapause during migration or post-migratory swarming phases. Species represented are: *Euploea core core*, *Euploea sylvester coreta*, *Tirumala limniace exoticus*, and *Tirumala septentrionis dravidarum*, *Danaus chrysippus*, *Euploea midamus rogenhoferi*, *Euploea mulciber mulciber*, *Tirumala septentrionis septentrionis*, *Acraea terpsicore*, *Byasa polyeuctes* and *Delias eucharis*.

Family	Migratory species	Migratory phase	Species	Sex and phase	F-statistic	df	R ² -value	p-value	y-intercept	Slope
Nymphalidae	yes	no	<i>E. c. core</i>	♀ OV	17.91	1,17	0.4844	0.0006	9.61	1.26
Nymphalidae	yes	yes	<i>E. c. core</i>	♀ RD	10.77	1,45	0.1752	0.0020	7.74	1.05
Nymphalidae	yes	yes	<i>E. c. core</i>	♂	168.00	1,70	0.7017	0.0000	-17.12	1.15
Nymphalidae	yes	no	<i>E. s. coreta</i>	♀ OV	19.12	1,19	0.4753	0.0003	-49.62	1.83
Nymphalidae	yes	yes	<i>E. s. coreta</i>	♀ RD	151.50	1,106	0.5845	0.0000	-21.38	1.33
Nymphalidae	yes	yes	<i>E. s. coreta</i>	♂	81.06	1,83	0.4880	0.0000	-6.62	0.96
Nymphalidae	yes	no	<i>T. l. exoticus</i>	♀ OV	2.61	1,7	0.1678	0.1500	111.29	0.42
Nymphalidae	yes	yes	<i>T. l. exoticus</i>	♀ RD	32.60	1,29	0.5130	0.0000	6.57	0.86
Nymphalidae	yes	yes	<i>T. l. exoticus</i>	♂	95.89	1,40	0.6983	0.0000	-6.06	0.8
Nymphalidae	yes	no	<i>T. s. dravidarum</i>	♀ OV	5.14	1,6	0.3714	0.0640	44.43	0.62
Nymphalidae	yes	yes	<i>T. s. dravidarum</i>	♀ RD	161.50	1,118	0.5742	0.0000	-7.72	1.08
Nymphalidae	yes	yes	<i>T. s. dravidarum</i>	♂	155.10	1,64	0.7033	0.0000	-14.55	0.98
Nymphalidae	no	no	<i>D. c. chrysippus</i>	♀	40.35	1,40	0.4897	0.0000	38.5	0.82
Nymphalidae	no	no	<i>D. c. chrysippus</i>	♂	47.81	1,48	0.4886	0.0000	-6.9	0.9
Nymphalidae	no	no	<i>E. m. rogenhoferi</i>	♀	11.44	1,6	0.7029	0.0000	3.29	1.21
Nymphalidae	no	no	<i>E. m. rogenhoferi</i>	♂	12.90	1,11	0.5342	0.0000	-61.78	1.37
Nymphalidae	no	no	<i>E. m. mulciber</i>	♀	55.40	1,22	0.5987	0.0148	-10.37	1.55
Nymphalidae	no	no	<i>E. m. mulciber</i>	♂	34.26	1,28	0.4978	0.0042	-42.09	1.21
Nymphalidae	no	no	<i>T. s. septentrionis</i>	♀	9.50	1,6	0.5484	0.0216	-19.06	1.63
Nymphalidae	no	no	<i>T. s. septentrionis</i>	♂	31.23	1,14	0.6683	0.0001	-40.96	1.11
Nymphalidae	no	no	<i>A. terpsicore</i>	♀	36.00	1,29	0.5385	0.0000	4.89	0.97
Nymphalidae	no	no	<i>A. terpsicore</i>	♂	20.55	1,19	0.4943	0.0002	4.99	0.79
Papilionidae	no	no	<i>B. p. polyeuctes</i>	♀	10.63	1,7	0.5463	0.0139	20.34	1.15
Papilionidae	no	no	<i>B. p. polyeuctes</i>	♂	20.56	1,15	0.5500	0.0004	-15.55	0.82
Pieridae	no	no	<i>D. eucharis</i>	♀	15.89	1,11	0.5537	0.0021	-22.9	1.86
Pieridae	no	no	<i>D. eucharis</i>	♂	14.39	1,22	0.3679	0.0010	1.59	0.8

Table 2. ANCOVA test statistics and p-values for the pair- and group-wise comparisons shown in Fig. 2, which are related to statistical summaries of individual species and sexes in Table 1. Differences in slopes are shown only when significant ($p < 0.05$).

Groups	Comparison of regressions of thorax and abdomen masses	Difference in	F statistic (ANCOVA)	df	p-value
<i>Euploea c. core</i>	♀ OV – ♀ RD	intercepts	6.06	1,1	0.0166
<i>Euploea c. core</i>	♀ OV – ♂	intercepts	58.70	1,1	0.0000
<i>Euploea c. core</i>	♀ RD – ♂	intercepts	16.37	1,1	0.0001
<i>Euploea c. core</i>	♀ OV – ♀ RD – ♂	intercepts	20.83	1,2	0.0000
<i>Euploea sylvester coreta</i>	♀ OV – ♀ RD	intercepts	8.80	1,1	0.0036
<i>Euploea sylvester coreta</i>	♀ OV – ♂	slopes	6.73	1,1	0.0109
<i>Euploea sylvester coreta</i>	♀ OV – ♂	intercepts	43.85	1,1	0.0000
<i>Euploea sylvester coreta</i>	♀ RD – ♂	slopes	5.64	1,1	0.0186
<i>Euploea sylvester coreta</i>	♀ RD – ♂	intercepts	31.09	1,1	0.0000
<i>Euploea sylvester coreta</i>	♀ OV – ♀ RD – ♂	slopes	4.63	1,2	0.0107
<i>Euploea sylvester coreta</i>	♀ OV – ♀ RD – ♂	intercepts	27.17	1,2	0.0000
<i>Tirumala limniace exoticus</i>	♀ OV – ♀ RD	intercepts	19.52	1,1	0.0000
<i>Tirumala limniace exoticus</i>	♀ OV – ♂	slopes	4.41	1,1	0.0001
<i>Tirumala limniace exoticus</i>	♀ OV – ♂	intercepts	72.17	1,1	0.0412
<i>Tirumala limniace exoticus</i>	♀ RD – ♂	intercepts	16.81	1,1	0.0000
<i>Tirumala limniace exoticus</i>	♀ OV – ♀ RD – ♂	intercepts	32.69	1,2	0.0001
<i>Tirumala septentrionis dravidarum</i>	♀ OV – ♀ RD	intercepts	2.25	1,1	0.0000
<i>Tirumala septentrionis dravidarum</i>	♀ OV – ♂	intercepts	21.95	1,1	0.1360
<i>Tirumala septentrionis dravidarum</i>	♀ RD – ♂	intercepts	36.05	1,1	0.0000
<i>Tirumala septentrionis dravidarum</i>	♀ OV – ♀ RD – ♂	intercepts	21.09	1,2	0.0000
<i>Danaus c. chrysippus</i>	♀ – ♂	intercepts	124.60	1,1	0.0000
<i>Euploea midamus rogenhoferi</i>	♀ – ♂	intercepts	4.77	1,1	0.0000
<i>Euploea m. mulciber</i>	♀ – ♂	intercepts	86.59	1,1	0.0425
<i>Tirumala. s. septentrionis</i>	♀ – ♂	intercepts	39.73	1,1	0.0000
<i>Acraea terpsicore</i>	♀ – ♂	intercepts	6.78	1,1	0.0000
<i>Byasa p. polyeuctes</i>	♀ – ♂	intercepts	30.86	1,1	0.0122
<i>Delias eucharis</i>	♀ – ♂	slopes	5.47	1,1	0.0000
<i>Delias eucharis</i>	♀ – ♂	intercepts	55.78	1,1	0.0255

It is unknown whether migratory danaines have differential investment in flight and reproductive tissue compared to other non-migratory danaines, or compared to non-danaine aposematic species. To test this, we compared thorax and abdomen masses of the four migratory danaine species from peninsular India with four non-migratory danaines (Fig. 2e–h, Table 2) and three non-migratory non-danaine aposematic species (Fig. 2i–k, Table 2). We also compared their relative investments in thorax and abdomen (Fig. 3, Table 3). This comparison showed that females of non-migratory danaines and non-migratory non-danaine aposematics also had heavier abdomens compared to males (Fig. 2, 3, Table 2, 3). Reproductive females of the three groups had similar thorax:abdomen ratios that differed substantially from danaine females in migratory phase and also with males of all species considered (Fig. 3c, Table 3). This pattern is generally true whether we compare these parameters in individual species (Fig. 2, 3a–b, Supplementary material Appendix 1 Table A2, A3), or whether the male and female thorax:abdomen ratios were compared across all species grouped by migratory habits (Fig. 3c, Kruskal–Wallis rank sum test: $\chi^2(4) = 29.267$, $p < 0.001$, followed by Dunn's tests in Table 3). Indeed, the range of data were consistent across all the species in terms of central tendencies and spread (Supplementary material Appendix 1 Table A2), i.e. the flight/reproductive morphology of all aposematic species is similar irrespective of their taxonomic affiliations (danaines versus non-danaines) or migratory behaviours

(migratory versus non-migratory species). However, our data revealed an interesting universal generality, that there are consistent differences among the sexes and among the reproductive phases of the females: females invested more in reproductive tissue compared to males, and reproductive females invested more in reproductive tissue compared to migrating and post-migratory swarming females in reproductive diapause, in both relative and absolute terms (Fig. 2, 3, Table 2, 3).

Discussion

Sex-specific strategies in resource allocation to different tissue are seen in many animals (Gäde 2002, Krasnov et al. 2003, Berwaerts et al. 2006). These differences result in discrete mass ratios of body parts, which may be interpreted as the relative investment in their respective functions. Our results show that females generally invested more in abdominal tissue than males even during reproductive diapause, which may affect various attributes related to flight performance such as take-off flight, wing beat frequency, and wing stroke amplitude (Berwaerts et al. 2002, 2006). This phenotypic dimorphism has far-reaching behavioural and evolutionary consequences for dispersal ecology. Female beetles, for example, are larger and have a higher flight capacity and higher inter-patch dispersal tendency than males, whereas males have a higher take-off tendency but lower inter-patch dispersal (Davis 1984).

Table 3. Test statistics and p-values for the pairwise comparisons shown in Fig. 3. Also shown are results of ANOVA for three-group comparisons between ovulating females, reproductive diapause females and males of the migratory danaine species. See Supplementary material Appendix 1 Table A2 for statistical summaries. Migratory danaines = *Euploea core core*, *Euploea sylvester coreta*, *Tirumala limniace exoticus*, and *Tirumala septentrionis dravidarum*, non-migratory danaines = *Danaus chrysippus chrysippus*, *Euploea midamus rogenhoferi*, *Euploea mulciber mulciber* and *Tirumala septentrionis septentrionis*, aposematics = non-danaine aposematic species, i.e. *Acraea terpsicore*, *Delias eucharis* and *Byasa polyeuctes polyeuctes*.

Groups	Thorax:abdomen ratio comparison pairs	Test statistic	p-value
<i>Euploea c. core</i>	♀ OV – ♀ RD	t = -4.12	0.0001
<i>Euploea c. core</i>	♀ OV – ♂	t = -8.46	0.0000
<i>Euploea c. core</i>	♀ RD – ♂	t = -3.95	0.0001
<i>Euploea c. core</i>	♀ OV – ♀ RD – ♂	F(2,135) = 26.4	0.0000
<i>Euploea sylvester coreta</i>	♀ OV – ♀ RD	t = -2.85	0.0041
<i>Euploea sylvester coreta</i>	♀ OV – ♂	t = -6.07	0.0000
<i>Euploea sylvester coreta</i>	♀ RD – ♂	t = -5.5	0.0000
<i>Euploea sylvester coreta</i>	♀ OV – ♀ RD – ♂	F(2,211) = 25.17	0.0000
<i>Tirumala limniace exoticus</i>	♀ OV – ♀ RD	t = -3.45	0.0021
<i>Tirumala limniace exoticus</i>	♀ OV – ♂	t = -6.32	0.0000
<i>Tirumala limniace exoticus</i>	♀ RD – ♂	t = -4.18	0.0000
<i>Tirumala limniace exoticus</i>	♀ OV – ♀ RD – ♂	F(2,79) = 23.61	0.0000
<i>Tirumala septentrionis dravidarum</i>	♀ OV – ♀ RD	t = -2.24	0.0265
<i>Tirumala septentrionis dravidarum</i>	♀ OV – ♂	t = -5.12	0.0002
<i>Tirumala septentrionis dravidarum</i>	♀ RD – ♂	t = -5.81	0.0000
<i>Tirumala septentrionis dravidarum</i>	♀ OV – ♀ RD – ♂	F(2,191) = 20.59	0.0000
<i>Danaus c. chrysippus</i>	♀ – ♂	W = 42.5	0.0000
<i>Euploea midamus rogenhoferi</i>	♀ – ♂	t = -2.85	0.0058
<i>Euploea m. mulciber</i>	♀ – ♂	W = 32	0.0000
<i>Tirumala. s. septentrionis</i>	♀ – ♂	t = -6.27	0.0000
<i>Acraea terpsicore</i>	♀ – ♂	W = 176.5	0.0028
<i>Byasa p. polyeuctes</i>	♀ – ♂	t = -6.14	0.0000
<i>Delias eucharis</i>	♀ – ♂	W = 15	0.0000
Migratory danaines	♀ RD – ♀ OV	z = 5.24	0.0000
Migratory danaines	♂ – ♀ OV	z = 9.49	0.0000
Migratory danaines	♂ – ♀ RD	z = 7.51	0.0000
Non-migratory danaines	♀ – ♂	z = -12.14	0.0000
Non-danaine aposematics	♀ – ♂	z = -7.43	0.0000
Migratory and non-migratory danaines	♀ OV – ♀	z = 1.93	0.5599
Migratory and non-migratory danaines	♀ RD – ♀	z = 8.76	0.0000
Migratory and non-migratory danaines	Migratory ♂ – non-migratory ♂	z = -0.49	1.0000
Migratory danaines and aposematics	Aposematic ♀ – ♀ OV	z = 0.2	1.0000
Migratory danaines and aposematics	Aposematic ♀ – ♀ RD	z = -4.83	0.0000
Migratory danaines and aposematics	Aposematic ♂ – migratory ♂	z = 0.30	1.0000
Non-migratory danaines and aposematics	Aposematic ♀ – danaine ♀	z = 2.10	0.3728
Non-migratory danaines and aposematics	Aposematic ♂ – danaine ♂	z = -0.08	1.0000

Tendency to migrate earlier or longer differs between the sexes and between juveniles and adults in organisms ranging from insects to mammals (Dobson 1982, Kjellén et al. 2001). This may be driven by a variety of reasons, such as competition for resources or mates, environmental conditions, and mating strategies (Perrin and Mazalov 2000, Hirota 2004). It is unknown whether the male-biased sex ratios in this study were because of female-biased mortality or difficulty of sampling females (perhaps because they fly higher than males and roost in the trees, out of reach of observers). For the Indian danaine populations, it remains to be seen whether sex- and age-dependent variation in migration propensity is present, and how this may relate to relative investments in flight and reproduction.

Lifetime resource allocation in holometabolous insects is shaped by multiple interrelated factors, such as larval feeding habits, resource type and availability, pupal development, adult life stage, and various life history traits (Marden 2000,

Boggs 2009). Nutrient distribution to different body parts is a dynamic process where relative investment in a single trait changes over an organism's lifetime, depending on its requirements and allometric constraints (Stjernholm et al. 2005, Mirth et al. 2016). In migratory species, for instance, larval reserves may be stored mostly as fat bodies before eclosion, while the adult butterfly's diet and reproductive tissue resorption may redirect nutrients to supplement its flight morphology, muscle repair, and other survival-related body functions. Conversely, adults may eclose with their long-distance flight apparatus adequately formed, with further feeding required to supplement their lipid stores. In fact, a combination of several alternative models of resource allocation may be necessary to explain the nutrient storage patterns of migratory insects, especially if combined with conditions of larval or adult food stress (Boggs 1994). Since even the mere presence of flight apparatus can reduce reproductive potential (Watt 1984, Dixon et al. 1993) and alter resource

allocation priorities (Zera and Larsen 2001) in wing-poly-morphic insect species, long-distance migration by flight may introduce significant tradeoffs between dispersal and reproduction (the oogenesis-dispersal syndrome; Johnson 1963; Bonte et al. 2012), especially in the case of female migratory danaines. However, elevated allocation or reallocation of resources to reproduction after migration may counteract the tradeoffs between dispersal and reproduction that occur before migration (Hughes et al. 2003, Stjernholm et al. 2005, Saastamoinen et al. 2010). On the other hand, pronounced flight muscle breakdown and resource reallocation to reproductive tissue (Stjernholm and Karlsson 2008) might also affect mating-related flight efficiency and oviposition. In the case of Indian danaines the feeding and resource allocation patterns in different life stages are unknown, but it appears that thoracic mass is not compromised even in the post-migratory reproductive phase. Whether the occurrence of a migratory phenotype has any influence on the lifetime fecundity of these butterflies compared to their non-migratory relatives remains to be examined.

Important environmental conditions such as food availability, the number of stopover sites and feeding opportunities, duration of active flight, and the direction of air currents determine flight efficiency and nutrient usage during a long-distance migration in insects (Brower et al. 2006, Åkesson and Hedenström 2007). Metabolic efficiency is of paramount importance in migrating species. In terms of energy output per gram wet mass, fat is by far the most effective energy store available to migrants, whether birds (Jenni and Jenni-Eiermann 1998) or insects (Beenackers et al. 1981, Arrese and Soulages 2010). This explains the relatively lighter abdomens of migrant females since they were filled primarily with fat bodies, whereas those of ovulating females also contained fully developed reproductive systems, spermatophores, and numerous ova. Elucidating the pathways of lipid and carbohydrate mobilization in the migratory and post-migratory swarming phases (Beenackers et al. 1984, Arrese and Soulages 2010) may explain age-dependent resource allocation and the switch from the migratory phenotype to the breeding phenotype (Cotto et al. 2014). Once reproductive diapause ends and breeding ensues, however, the foraging pressure on female butterflies may be somewhat assuaged by nutritional contributions from males through the spermatophore (Karlsson 1995). Radiotracing studies would help understand how these resources are accumulated and differentially utilized (Boggs 1997), whether the utilization patterns undergo significant changes depending on migratory behaviour and phase, and what environmental or developmental factors might regulate the relevant biochemical pathways.

Finally, resource allocation at the intersection of migration and unpalatability is an interesting subject that deserves some consideration. Palatable, unpalatable, and mimetic species have distinct flight morphologies (Cook et al. 1969, Srygley and Chai 1990, Marden and Chai 1991), variously influenced by predator-prey interactions, wing colour polymorphisms, and evasive flight patterns. Aposematic

butterflies are able to afford a relatively higher resource allocation to their lifetime reproductive efforts, and thus have relatively heavier abdomens compared to palatable species (Marden and Chai 1991). However, this also puts a physical constraint on their flight capabilities, especially flight speeds and escape manoeuvres, which is especially true for females (Marden 2000). Interestingly, all the aposematic species in our study invested similarly in flight and reproduction when grouped by sex, and the only significant difference was in the female migratory phase. It may be argued that even if males and females invest differentially in flight and reproductive tissue, sex-specificity in these investments results merely from phylogenetic constraints within danaine butterflies. However, similar relative investments in flight and reproductive tissue in other aposematic species from three different families (Nymphalidae, Papilionidae, and Pieridae; Fig. 2, 3) suggests that there is an optimal thorax:abdomen ratio for aposematic species on which multiple species from different clades have converged.

We showed that migratory behaviour affects the two sexes differentially, females being much more sensitive to switches in reproductive versus migratory phases with respect to relative investment in reproduction and flight morphology. However, they do this by modulating their investment in reproductive tissue alone, not by changing investment in flight muscles. These patterns were consistent across migratory and non-migratory populations of the same species, in related non-migratory species, as well as in unrelated aposematic species, revealing a very broad generality of sexual differences in the reproductive effort and relative investments in thorax and abdomen. These results elucidate the sexually dimorphic morphological – and by extension physiological – adaptations to migratory behaviour in insects, and underscore a notable sex-specific morphological polyphenism in resource allocation that is relevant to developmental phases in the lives of migratory species.

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Supplementary material (available online as Appendix-oik-04593 at <www.oikosjournal.org/appendix/oik-04593>). Appendix 1.