



## Consistent behavioural differences between migratory and resident hoverflies



Jannic Odermatt<sup>a</sup>, Joachim G. Frommen<sup>b</sup>, Myles H. M. Menz<sup>a, c, \*</sup>

<sup>a</sup> Department of Community Ecology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

<sup>b</sup> Department of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Hinterkappelen, Switzerland

<sup>c</sup> School of Plant Biology, The University of Western Australia, Crawley, WA, Australia

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Many animals differ consistently in the way they behave across time and context. This animal personality has been linked to traits such as life history strategies or dispersal. However, few studies have addressed the relationship between consistent behavioural differences and migration. This is of particular interest with respect to partial migration, in which only part of a population migrates while the other remains resident. We investigated whether two behavioural traits (activity and stress response) are consistent across time in individuals of two partially migratory hoverfly species, *Episyrphus balteatus* and *Scaeva selenitica*. We also investigated whether there were consistent behavioural differences between migratory and resident flies within species. Individual activity was consistent across time in both species. Additionally, activity of female *E. balteatus* differed between the phenotypes, with summer insects being more active than migrating and overwintering individuals in our assays. Furthermore, females of *S. selenitica* were more active and less easily stressed than *E. balteatus*. The results not only highlight that hoverflies behave consistently across time, but also that behavioural differences also occur between migratory and resident flies. They also provide evidence for the possible role of behavioural differences in influencing partial migration decisions within populations.

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Migration is a ubiquitous and fascinating phenomenon, encompassing a wide array of taxa from zooplankton and insects to fish, birds and mammals (Dingle & Drake, 2007). The broad implications and interactions with physiology, behaviour, population structure and ecosystem dynamics have been the basis for a significant body of research (see Bauer & Hoye, 2014; Berthold, 2001; Dingle & Drake, 2007). Within many migratory species, a proportion of a population may overwinter in the breeding grounds instead of migrating, termed partial migration (Chapman, Brönmark, Nilsson, & Hansson, 2011). Partial migration is often viewed as a transitional state between complete migration and residency (Berthold, 2001), yet the mechanisms influencing partial migration are still poorly understood. Studying the physiological, morphological or behavioural differences between migratory and resident phenotypes could provide important clues to better understand the phenomenon of partial migration (Chapman, Brönmark, et al., 2011).

Investigating the mechanisms behind partial migration may require a bottom-up process, starting with the individual level: what causes an individual to stay or alternatively, what triggers migration (Chapman, Brönmark, et al., 2011)? Understanding these proximate processes may elucidate the evolution of migration itself and allow us to see how populations respond to environmental changes. For example, Ducatez et al. (2012) demonstrated that the butterfly *Pieris brassicae* exhibited correlated mobility-related traits and behavioural, physiological and morphological features that were consistent across time, which they summarized as a mobility syndrome. Ultimately, however, it is the behavioural response that dictates the fate of the individual, even if physiology may strongly influence or limit the behaviour. Derived from this, differences in dispersal or even migration could be mediated by consistent personality traits.

Individuals in a wide array of different taxa differ consistently in the way they behave under the same given stimuli (e.g. Bell, Hankison, & Laskowski, 2009; Kralj-Fišer & Schuett, 2014). If this consistent behavioural variation between individuals persists across time and context, this is considered as animal personality (e.g. Dingemanse, Kazem, Réale, & Wright, 2010; Schuett et al., 2011) or coping styles (Koolhaas et al., 1999). Animal personality

\* Correspondence: M. Menz, Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland.

E-mail address: [myles.menz@iee.unibe.ch](mailto:myles.menz@iee.unibe.ch) (M. H. M. Menz).

needs to be described as measurable traits, such as aggressiveness or activity. Personality traits are often consistent across the whole ontogeny, even with major life stage transitions, such as metamorphosis (e.g. Gyuris, Feró, & Barta, 2012). Intriguingly, these differences in personality have been shown to be linked to a multitude of individual traits, such as life history strategies (Wolf, van Doorn, Leimar, & Weissing, 2007), dispersal (Cote, Clobert, Brodin, Fogarty, & Sih, 2010), metabolic rate (Terracciano et al., 2013), sexual selection (Montiglio, Wey, Chang, Fogarty, & Sih, 2016) or invasive capacity (Réale & Dingemanse, 2012; Suarez, Tsutsui, Holway, & Case, 1999).

Although consistent behavioural variation and its implications are widely discussed, research is still mainly focused on vertebrate species, which only represent 2% of the total global number of animal species (Kralj-Fišer & Schuett, 2014). In contrast, invertebrates are highly underrepresented in personality studies. In a recent review Kralj-Fišer and Schuett (2014) found 47 empirical studies assessing invertebrate personality variation, compared to almost 4000 for vertebrates. This discrepancy is surprising, as the short generation time of many invertebrates makes them highly suitable to study genetically and environmentally induced differences in behavioural phenotypes. Furthermore, many insect species offer some completely different life histories. These life history traits and their potential for personality studies are particularly well reflected in several noteworthy results such as the different personalities of clonal *Acyrtosiphon pisum* aphids (Schuett et al., 2011), consistent behavioural variation of social insects within and between colonies (Chapman, Thain, Coughlin, & Hughes, 2011; Jandt et al., 2014) or consistent personality traits across ecdysis in *Pyrhocoris apterus* fire bugs (Gyuris et al., 2012). Similarly, we might predict the presence of consistent behavioural differences between phenotypes in partially migratory populations.

Bridging the gap between consistent personality traits and partial migration might be one step towards understanding this intriguing phenomenon. To our knowledge, there have been relatively few studies linking behavioural traits to migratory state (Chapman, Hulthén, et al., 2011; Mettke-Hofmann, Ebert, Schmidt, Steiger, & Stieb, 2005; Nilsson, Nilsson, Alerstam, & Bäckman, 2010), and these have primarily involved vertebrates. For example, Chapman, Hulthén, et al. (2011) showed that bolder roaches, *Rutilus rutilus*, are more likely to migrate, whereas Nilsson et al. (2010) showed that migratory blue tits, *Cyanistes caeruleus*, were less neophobic than nonmigratory individuals. Interestingly, despite insects being the most abundant and important group of terrestrial migrants (Chapman et al., 2012; Holland, Wikelski, & Wilcove, 2006; Hu et al., 2016), their migration behaviour is relatively poorly studied compared to vertebrates in general (Chapman, Reynolds, & Wilson, 2015).

Here we investigated the consistency in behaviour over time in individuals of two migratory species of hoverfly (Diptera, Syrphidae), *Episyrphus balteatus* and *Scaeva selenitica*, in relation to their migratory status. Hoverflies are known for their important role in ecosystem services such as pollination and biological control of aphids (e.g. Hondelmann & Poehling, 2007; Jauker, Bondarenko, Becker, & Steffan-Dewenter, 2012). While most European hoverfly species overwinter in the summer quarters as preimaginal stages (Keil, Dziöck, & Storch, 2008), others migrate southwards in autumn (see Aubert, Aubert, & Goeldlin, 1976; Gatter & Schmid, 1990; Fig. 1) to spend the winter in southern Europe and the Mediterranean (Hondelmann & Poehling, 2007; Raymond, Sarthou, et al., 2014; Raymond, Vialatte & Plantagenest, 2014). Hoverflies can be observed migrating south in large numbers in autumn, particularly at locations where migrating swarms are funnelled by the topography, such as southern Scandinavia (Goeldlin de Tiefenau, 1981) and high altitude passes in the Alps (Aubert et al.,

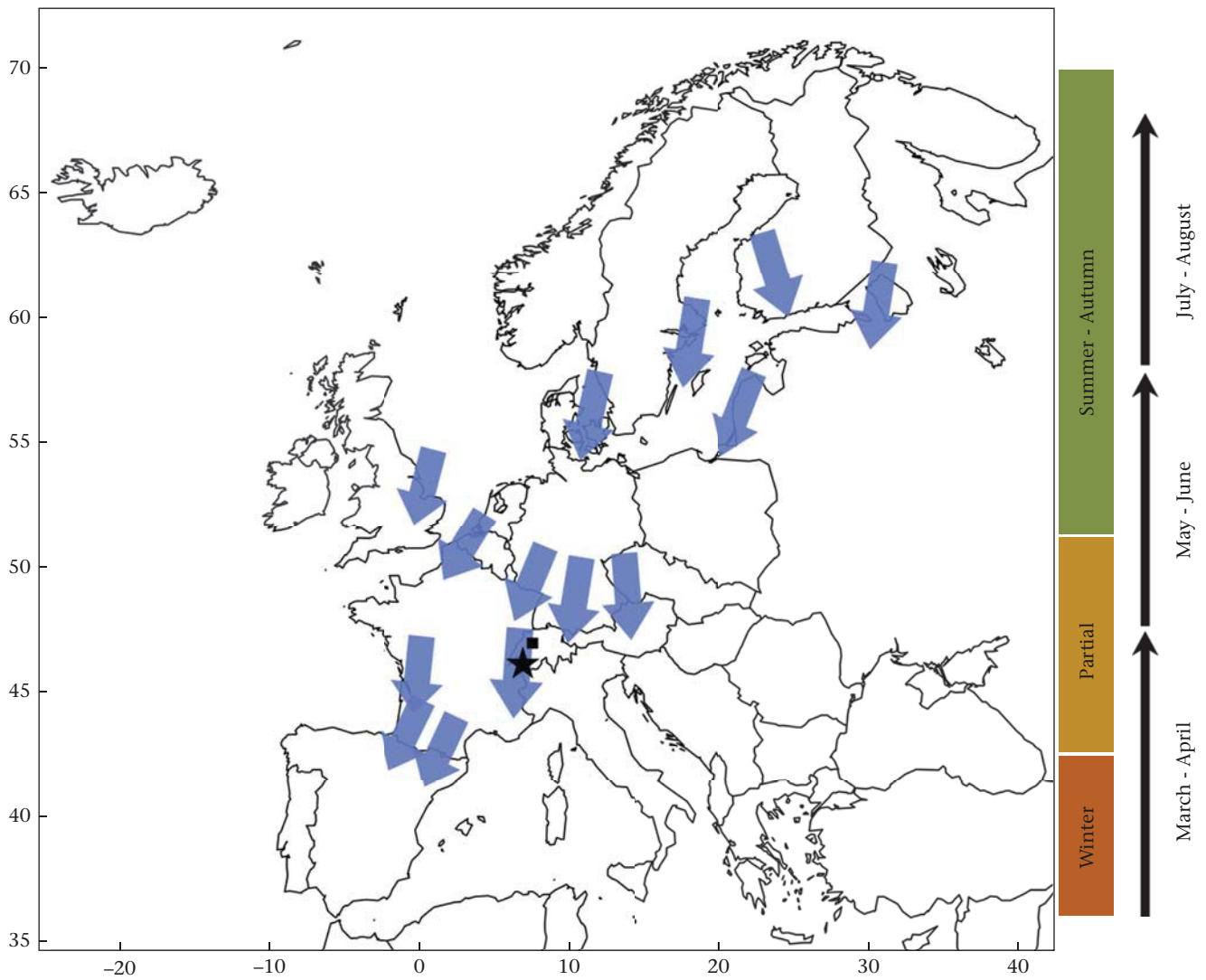
1976) and the Pyrenees (Lack & Lack, 1951; Fig. 1). Within their wintering habitat, several species are also thought to reproduce (Raymond, Sarthou, et al., 2014). The offspring of the autumn migrants are then thought to make the return northward migration in spring. However, little is known regarding the spring migration, which has rarely been observed, but it is thought to occur in a multigenerational, gradual pattern (Raymond, Vialatte, et al., 2014; Fig. 1) as is observed in other long-distance migrant insects, such as butterflies (e.g. Inamine, Ellner, Springer, & Agrawal, 2016; Stefanescu et al., 2013). Furthermore, within populations of migratory hoverfly species, some individuals in some regions may overwinter as adults in the summer quarters, typically fertilized females in reproductive diapause (Hondelmann & Poehling, 2007; Raymond, Sarthou, et al., 2014; Rotheray & Gilbert, 2011). Both strategies, migration and overwintering, are associated with large expenditure, risk and reproductive potential (Chapman, Brönmark, et al., 2011). This phenomenon is often described as bet hedging, where the likelihood of increased fitness from one strategy is offset against a reduced reproductive output from another (Hopper, 1999; Tomlinson & Menz, 2015). Therefore, both strategies ought to be under strong selection but, so far, no genetic dissimilarities have been found between migratory hoverflies from different geographical regions (Hondelmann, Borgemeister, & Poehling, 2005; Francuski, Djurakic, Ståhls, & Milankov, 2014; Raymond, Plantagenest & Vialatte, 2013) or the different phenotypes (Raymond, Plantagenest, Gauffre, Sarthou & Vialatte, 2013). Furthermore, there are no apparent differences in wing morphology between migratory and overwintering phenotypes (Raymond, Vialatte, et al., 2014). This led us to hypothesize that other more cryptic differences may be driving partial migration in hoverflies, such as physiology or personality (Tomlinson & Menz, 2015). Thus far, nothing is known about consistent differences in the behaviour of migrating and overwintering hoverflies. Accordingly, it remains elusive how these differences would be reflected in differences in migratory and resident individuals. To fill this gap in our knowledge, we examined the following questions. (1) Do individual hoverflies behave consistently over several days? (2) Does the behaviour of migrating individuals differ consistently from that of overwintering individuals? (3) Are there major behavioural differences between two migratory species?

## METHODS

### Study Species

*Episyrphus balteatus* and *Scaeva selenitica* (Fig. 2) are two common hoverfly species that are widespread in Central Europe (Speight, 2014). Both species feed on aphids as larvae and pollen and nectar as adults. Although wild populations may survive longer, evidenced by the overwintering females, the average longevity in the laboratory is 7–9 days, depending on the food type (Pinheiro, Torres, Raimundo, & Santos, 2013), with *S. selenitica* generally living longer (J. Odermatt, personal observation). *Scaeva selenitica* is larger (total body length, mean  $\pm$  SE; females:  $12.78 \pm 0.41$  mm,  $N = 19$ ; males:  $13.93 \pm 0.85$  mm,  $N = 4$ ) than *E. balteatus* (females:  $10.55 \pm 0.14$  mm,  $N = 44$ ; males:  $10.43 \pm 0.08$  mm,  $N = 97$ ).

All flies tested were collected from the field as adults and assigned to one of the following three phenotypes: overwintering, summer and migrating. Resident (overwintering and summer) flies were collected in Bern, Switzerland ( $46^{\circ}56'38''N$ ,  $7^{\circ}26'49''E$ ; Fig. 1) from April to August 2015. Individuals designated as summer flies were those collected between May and July. Only females captured in April were designated as overwintering. In May, the first males were caught, suggesting that overwintering and spring-emerging



**Figure 1.** Schematic representation of the migration of hoverflies in Europe. The blue arrows indicate general flight directions during the southward migration in autumn, from locations where hoverflies have been observed migrating (centre points of the arrows). The migration directions presented include observations from multiple species. The northward arrows on the right of the figure represent the hypothesized multigenerational return migration in the spring, and the months in which the flies probably travel north. This represents the predicted scenario for species such as *Episyrphus balteatus*. The area where migratory hoverflies are thought to overwinter is indicated in dark orange. The area where flies may overwinter (partially migratory populations) is indicated in light orange, and represents our prediction of where most partial migrants may occur. The summer distribution of *E. balteatus* is indicated in green. The star indicates the study site at Col de Bretolet in the Swiss Alps and the square indicates the city of Bern. The locations and directions of the autumn migration have been redrawn and adapted from Goeldlin de Tiefenau (1981) and Torp Pedersen (1984).

females could no longer be clearly separated, as males tend not to overwinter (Rotheray & Gilbert, 2011). Migrating flies were collected in August and September 2015 at Col de Bretolet (46°08'30"N, 6°47'44"E), an alpine pass at 1923 m above sea level in the Swiss Alps, approximately 100 km southwest of Bern (Fig. 1). Migrating flies were captured on the wing as they were flying southwest through the pass.

Flies were measured and individually colour marked on the thorax, using felt-tip pens, after 10 min in the refrigerator to facilitate handling. The flies were kept in mesh cages of 0.5 × 0.5 m and 1 m high in a controlled temperature room at 20 °C and 16:8 h light:dark. Flies were kept in small groups of three to seven individuals per cage. Food (commercially available bee-collected pollen) and water (cotton wool moistened with 20% (w/w) glucose solution) were provided ad libitum in three petri dishes, which were checked and refilled daily.

#### Behavioural Tests

The flies were acclimatized to laboratory conditions for 2–3 days before any behavioural tests. Thereafter, behaviour was assessed using two different tests, which were each conducted four times, resulting in a total of eight test runs per individual. All behavioural tests were performed on 2 days, 3–4 days apart. The test procedures consisted of a stress and an activity test. Both test procedures were run twice per test day, once in the morning between 0830 and 1200 hours, and once in the afternoon between 1230 and 1600 hours. In the morning, before any tests were made, each fly was weighed and tested for flying ability. If there was any damage to the wings, or the fly was otherwise not able to fly, the individual was excluded from any further testing. The two tests were conducted consecutively in a random order, with a break of 2 min before and between the tests. This procedure was repeated in



**Figure 2.** (a) Male *Episyrphus balteatus* and (b) male *Scaeva selenitica*. The black scale bars represent 1 cm. Photographs: L. Dällenbach.

the afternoon. The cages and the individual within the cage were randomly selected for each series of tests, by rolling a dice. All behavioural tests were performed in a controlled temperature room different to where the flies were kept, but with the same temperature and light conditions (20 °C, 16:8 h light:dark).

#### Activity Test

In the first behavioural test, we tested the individual's activity by placing it in a small transparent circular arena (30 cm diameter and 33 cm high). We recorded the time spent flying or walking for 10 min, following an initial 1 min of acclimatization. The test was filmed using a video camera positioned above the arena (see [Supplementary material, Videos 1 and 2](#)). The times spent flying and walking were significantly correlated (Pearson correlation: all  $r \geq 0.49$ ,  $P \leq 0.001$ ); therefore, these measures were combined into a single activity value. All videos were analysed with the program Jwatcher\_V1.0 (<http://www.jwatcher.ucla.edu>).

#### Stress Test

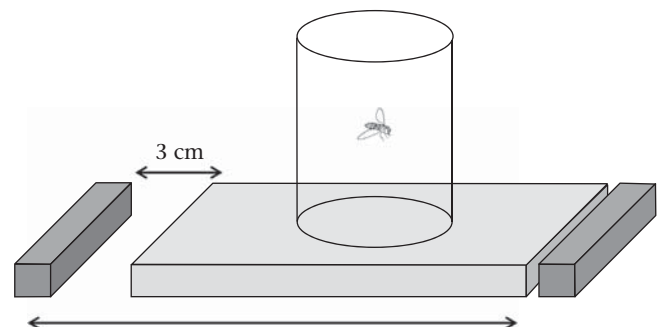
During this test, grooming behaviour was interrupted by moving the cage, causing the fly to stop grooming. Self-grooming is considered important for body integrity, for instance as protection against pathogens ([Yanagawa, Guigue, & Marion-Poll, 2014](#)) or for maintaining olfactory accuracy of the antennae ([Böröczky, Wada-](#)

[Katsumata, Batchelor, Zhukovskaya, & Schal, 2013](#)), and thus we considered disturbances to grooming to be stressors. The test individual was placed in a vial and gently sprayed on its back with water. This procedure readily elicits self-grooming behaviour. After spraying, the hoverfly was placed in a transparent test arena (10 cm diameter and 15 cm high; [Fig. 3](#)), fixed on a mobile board. The test was initiated after 30 s of acclimatization. As soon as the fly showed grooming behaviour for longer than 3 s, the mobile board was moved horizontally for 3 cm ([Fig. 3](#)), inducing a cessation of grooming. We then measured the time until grooming resumed (stress latency). This test was repeated up to five times for a total of 10 min. After a measured grooming event and the following disturbance, further grooming events were ignored for 20 s to let the fly return to normal activity. If a fly did not show any grooming behaviour despite the initial water spray, it was sprayed again after 4 min. If the fly still did not demonstrate any grooming behaviour, it was left in the cage until the end of the 10 min test period. The period of inactivity after the induced stress was from 0 (no reaction to the disturbance at all) up to several minutes in some cases. To account for the possibility that long periods of inactivity represented a lack of response instead of stress, we stopped measuring after 5 s, if the fly did not move. Therefore, all stress values range from 0 to 5 s. Values of 0 represent continuous grooming or moving. The measurements were averaged for each test run, resulting in four mean stress responses per individual, for each of the four test times.

#### Statistical Analyses

##### Consistency of behaviour across time

We tested whether the behaviour of individual flies was consistent across time for two different behavioural assays, activity and stress latency. Each of the two assays was repeated four times. All statistical analyses were done in R version 3.23 ([R Core Team, 2015](#)). First, we analysed the individual repeatability of the responses to the stress and the activity tests. This was done with a repeatability test using the rptR package ([Nakagawa & Schielzeth, 2010](#)). Zero values can be interpreted in two ways: first, they can indicate extremely fast reaction times or extremely low activity; second, they might indicate that the individual did not react as expected to the treatment. Therefore, we initially excluded zero values from the repeatability analyses (activity: 158 of 359 individual tests; stress: 15 of 301 tests). Activity data for *E. balteatus* were log transformed and stress latency data for *S. selenitica* were square root transformed so that the residuals visually indicated a normal distribution. Repeatability was calculated adhering to a normal distribution, using a generalized linear mixed-effects model



**Figure 3.** The experimental set-up for the stress test. The fly received a small water spray on its back before it was placed in the cylindrical plastic test arena. When the fly began to groom, the mobile board (light grey) was moved horizontally to interrupt the grooming behaviour.

(bootstrapping = 1000), with individual as the grouping factor. Only individuals that completed at least three of the test runs were used to analyse repeatability. To determine whether excluding zero values would influence repeatability, we also calculated Kendall's *W* coefficient of concordance (Kendall, 1948) including all data, using the irr package (Gamer, Lemon, Fellows, & Singh, 2012). *W* = 1 indicates complete concordance. Here, the data did not need to follow a specific distribution and thus no transformation was necessary. Kendall's *W* coefficient of concordance can also be considered as 'broad sense repeatability' (Gyuris et al., 2012).

Comparison of behaviour between sexes

All subsequent analyses between groups (sex, phenotypes and species) were compared using linear mixed-effects models (LMM) using the package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Individual was included as a random factor in the models to account for multiple testing. Residuals of the models were visually checked for conformity to a normal distribution. Activity data were square root transformed to adhere to model assumptions. Significance of the explanatory variable (sex) was tested using a likelihood ratio test (Elman & Hill, 2009).

Comparison of behaviour between phenotypes

We tested whether behaviour differed between the phenotypes within species using LMMs. As females are the predominant sex found overwintering and in migrating swarms (Hondelmann & Poehling, 2007), only females were used for this analysis. Furthermore, we only conducted this analysis on *E. balteatus*, as we did not catch any migrating individuals of *S. selenitica*. Female *E. balteatus* collected in April were assigned to the overwintering group, those from May to August were assigned to the summer population, and individuals caught on active migration in the Swiss Alps in August and September were assigned to the migrating group.

Comparison of behaviour between species

Only females were used for the comparison between species. Activity and stress of *E. balteatus* and *S. selenitica* females were compared using LMMs, including all females used in the study. Significance of the explanatory variable (species) was tested using a likelihood ratio test.

Ethical Note

There are no legal requirements for the use of hoverflies in research conducted in Switzerland. However, we took extra care to maintain the flies under good conditions in the laboratory, as described above.

RESULTS

Consistency of Behaviour Across Time

Not all individuals accomplished the full eight test runs. Of the original 141 *E. balteatus* [44 females (eight migrating, 20 overwintering, 16 summer) and 97 males (one migrating, 96 summer)], 50 died before the second test day [13 females (one migrating, 10 overwintering, two summer) and 37 males (summer)], reducing the sample size to 91 (31 females and 60 males). Of the original 23 *S. selenitica* (19 females and four males), two overwintering females died, resulting in 21 (three female and four male summer, 14 female overwintering) that completed at least three tests. While the survival rate seems relatively low, this is in accordance with the average longevity of hoverflies previously observed in laboratory conditions (Pinheiro et al., 2013). Owing to the low number of *S. selenitica* males (*N* = 4), only females were analysed further.

The results of the two consistency tests (repeatability and Kendall's *W*) were generally in agreement, despite the exclusion of zero values in activity for the repeatability test (Table 1). Here we focus on the results of the repeatability tests (Nakagawa & Schielzeth, 2010). In *E. balteatus*, activity was consistent across days for males and females (Table 1), whereas the pattern was less clear for stress. Male *E. balteatus* showed a significantly consistent stress latency over time, while females did not (Table 1). In contrast, repeatability in female *S. selenitica* was significant for both activity and stress tests (Table 1).

Behavioural Differences Between Sexes

In *E. balteatus*, the sexes differed significantly for the stress test, with the latency between the disturbance and recommencement of grooming being longer for females (mean ± SE = 3.02 ± 0.12 s) than for males (2.45 ± 0.09 s; Table 2). There was no significant difference in activity between the sexes for *E. balteatus* (Table 2).

Behavioural Differences Between Phenotypes

There was a significant difference in activity between the phenotypes in *E. balteatus*. The overwintering (mean ± SE = 48.08 ± 11.52 s) and migrating (11.2 ± 3.92 s) females were significantly less active than the summer females (summer: 84.91 ± 16.76 s; Fig. 4a, Table 3). In contrast, there were no significant differences in stress latency between the phenotypes (Fig. 4b, Table 3).

Comparison of Behaviour Between Species

The females of the two species differed significantly in their behaviour. Wintering females of *S. selenitica* were significantly

Table 1 Results of the behavioural consistency tests

Behavioural assay	<i>Episyrphus balteatus</i>						<i>Scaeva selenitica</i>							
	Repeatability				Kendall's <i>W</i>		Repeatability				Kendall's <i>W</i>			
	<i>R</i>	95% CI	<i>P</i>	<i>N</i>	<i>W</i>	<i>P</i>	<i>R</i>	95% CI	<i>P</i>	<i>N</i>	<i>W</i>	<i>P</i>	<i>N</i>	
Activity males	0.255	0, 0.483	0.027	25	0.34	<b>0.039</b>	56							
Activity females	0.29	0, 0.553	0.037	13	0.373	<b>0.043</b>	30	0.391	0.072, 0.635	< <b>0.001</b>	15	0.579	<b>0.002</b>	17
Stress latency males	0.354	0.184, 0.51	< <b>0.001</b>	47	0.397	0.019	32							
Stress latency females	0.183	0, 0.369	0.113	27	0.35	0.124	18	0.726	0.491, 0.855	< <b>0.001</b>	18	0.742	< <b>0.001</b>	16

Each of the two behavioural assays (activity and stress latency) were conducted four times for each individual. Repeatability was calculated adhering to a normal distribution, using a generalized linear mixed-effects model, excluding zero values. *P* values were calculated using likelihood ratio tests. Kendall's *W* was calculated including zero values. Significant differences (*P* < 0.05) are presented in bold.

**Table 2**

Results of linear mixed-effects models comparing activity and stress latency between the sexes within species and between species

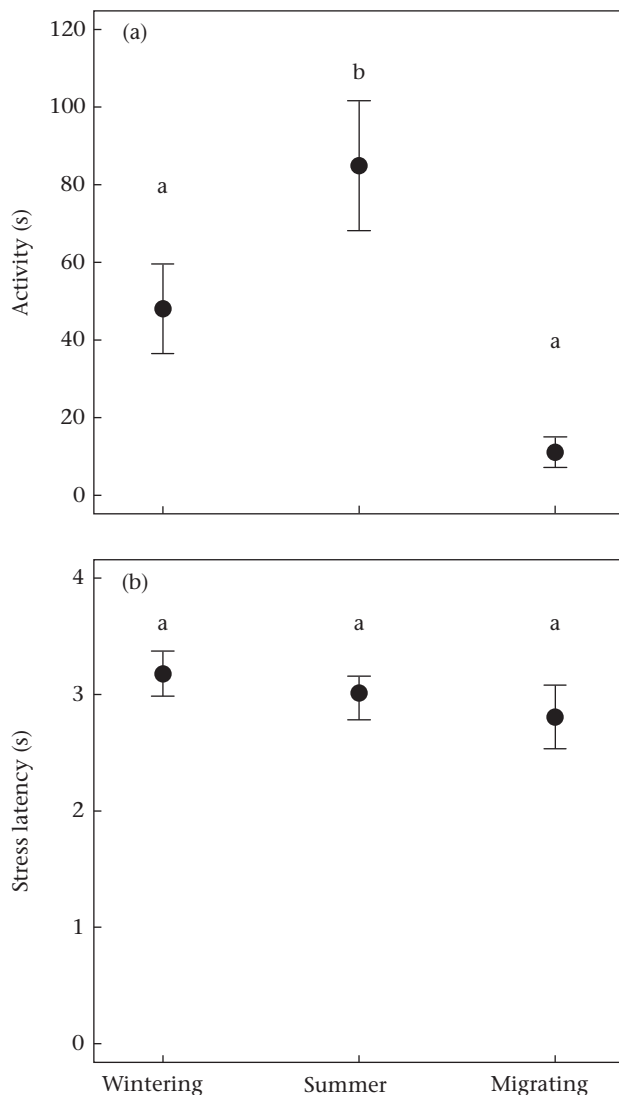
	Activity (square root)					Stress latency				
	LRT	<i>P</i>	ΔAIC	Est.	SE	LRT	<i>P</i>	ΔAIC	Est.	SE
<i>E. balteatus</i> (Sex)	3.20	0.074	1.2	−1.180	0.661	<b>8.84</b>	<b>0.003</b>	−6.9	−0.587	0.195
Species	<b>23.84</b>	<b>&lt;0.001</b>	−21.9	10.86	1.271	<b>19.91</b>	<b>&lt;0.001</b>	−17.9	−1.872	0.368

Only wintering females were used for the comparison between species. *P* values are based on likelihood ratio tests (LRT, Chi). Est. is the model estimate. ΔAIC is the change in Akaike's information criterion between the models with and without the explanatory variable (Sex or Species). Significant LRT (*P* < 0.05) results are presented in bold. Activity data were square root transformed.

more active (mean ± SE = 275.79 ± 25.5 s, *N* = 14) than *E. balteatus* (48.08 ± 11.52 s, *N* = 20; Fig. 5a, Table 2). The latency between disturbance and grooming activity (stress) was significantly shorter for wintering female *S. selenitica* (1.22 ± 0.15 s, *N* = 14) than *E. balteatus* (3.18 ± 0.19 s, *N* = 20; Fig. 5b, Table 2).

## DISCUSSION

In this study, we demonstrated that activity of individual hoverflies is consistent across time. Furthermore, we showed



**Figure 4.** Comparison of (a) activity and (b) stress latency (means ± SE) between the phenotypes in female *Episyrphus balteatus*. Lowercase letters indicate significant differences at *P* < 0.05.

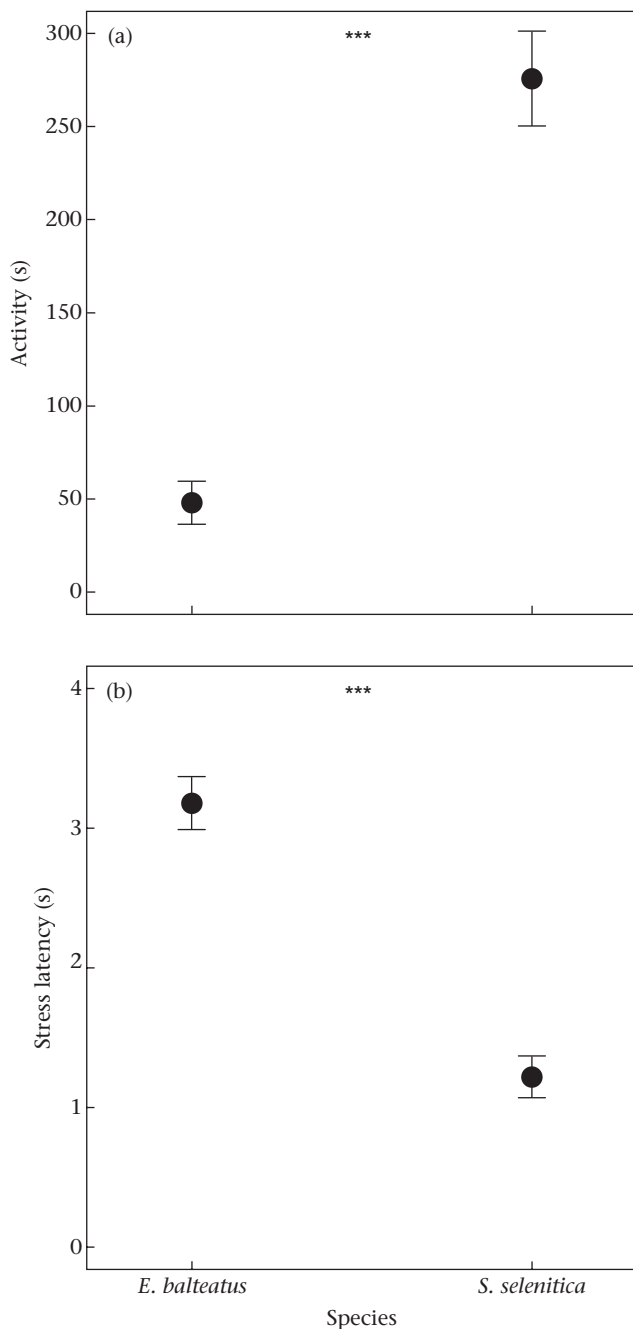
significant differences in activity between phenotypes of *E. balteatus*, with migrants and overwintering flies being less active than summer individuals. This is in agreement with previous studies demonstrating consistent behavioural traits in other insects (e.g. Bell et al., 2009; Brodin, 2009; Chapman, Thain, et al., 2011; Gyuris et al., 2012; Gyuris, Feró, Tartally, & Barta, 2011) and our recorded repeatability values are within or greater than the range typically reported for invertebrates (Bell et al., 2009).

Our results show a significant behavioural difference between migrating, overwintering and summer females of *E. balteatus*. Previous studies have found behavioural differences between migratory and resident phenotypes in fish, birds and insects (e.g. Chapman, Hulthén, et al., 2011; Nilsson et al., 2010; Zhu, Gegeer, Casselman, Kanginakudru, & Reppert, 2009). In our study, activity differed between the seasons and the phenotypes, whereas in the case of stress, there was no difference. Counterintuitively, the migratory individuals were the least active, while the summer ones were the most active. This could allude to an energy-conserving state in the migrants, if they cannot continue their migration, which probably involves significant energy expenditure, as is the case with birds (Wikelski et al., 2003). Overwintering female hoverflies enter a state of reproductive diapause, which is marked by an accumulation of energy reserves, such as growth of the fat bodies (Hondelmann & Poehling, 2007). Unfortunately, no physiological studies have investigated the energy requirements for migrating hoverflies. However, Attisano, Tregenza, Moore, and Moore (2013) demonstrated significant physiological differences between migrant and resident female milkweed bugs, *Oncopeltus fasciatus*. Furthermore, the migrating flies may not have received the necessary stimuli to trigger them to make a departure decision or continue migrating (see Bauer et al., 2011; Chapman et al., 2015; Drake & Reynolds, 2012), such as temperature changes (Wikelski et al., 2006) or favourable winds (Chapman, Reynolds, Hill, et al., 2008; Chapman, Reynolds, Mouritsen, et al., 2008; Mikkola, 2003). While there is no information available on what triggers migratory behaviour in hoverflies, studies on other insects may shed some light on the mechanisms behind this. For example, dragonflies (*Anax junius*) have been shown to initiate migration following a drop in night-time temperatures in autumn (Wikelski et al., 2006). If hoverflies follow similar cues, maintaining them at

**Table 3**Estimates from linear mixed-effects models comparing stress latency and activity between phenotypes of female *Episyrphus balteatus*

Comparison	Estimate	SE	<i>P</i>
<b>Stress latency</b>			
Summer versus Overwintering	0.180	0.318	0.575
Migrating versus Summer	0.136	0.399	0.736
Migrating versus Overwintering	0.316	0.397	0.413
<b>Activity (square root)</b>			
Summer versus Overwintering	2.499	1.167	<b>0.039</b>
Migrating versus Summer	4.519	1.443	<b>0.003</b>
Migrating versus Overwintering	2.020	1.417	0.162

Significant differences (*P* < 0.05) are presented in bold. Activity data were square root transformed.



**Figure 5.** Comparison of (a) activity and (b) stress latency (means  $\pm$  SE) between wintering females of *Episyrrhus balteatus* and *Scaeva selenitica*. \*\*\* $P < 0.001$ .

20 °C in the laboratory may have led them to cease migratory behaviour. Additionally, providing them with food and water ad libitum may have simulated a favourable environment and reduced activity. Therefore, it may be important to conduct future studies under field conditions, preferably close to sites where flies are undertaking active migration.

Two conspicuous patterns were embedded in our results. First, consistency in *E. balteatus* depicted a trend of lower repeatability than in *S. selenitica*, despite fewer tested individuals in the latter species. Either *E. balteatus* has more behavioural phenotypic plasticity, which consequently resulted in a lower repeatability, or the laboratory conditions affected *E. balteatus* more than *S. selenitica*, by making them less responsive. Second, only the males were consistent in the stress test across time. Here, we can only hypothesize. The

season might have played a role, as the migrating and the overwintering flies were almost exclusively females. It is conceivable that, during migration, responses to disturbance are less consistent. Alternatively, consistency in stress responses might be favoured in males, which might be reflected in the generally lower stress response of males in *E. balteatus*. The summer is dominated by reproductive behaviour when males occupy temporary territories and hover in sunny places waiting for females. A lower stress response may be advantageous when responding to disturbances within the territories, such as the presence of other males or females.

Finally, our results show that even ecologically similar species may differ greatly in their behavioural responses. These differences between species could be based on other factors, such as body size. For example, *S. selenitica* is heavier and larger than *E. balteatus* and consequently might need to be more selective about potential oviposition sites, resulting in a more active behaviour. The larvae of *S. selenitica* are also larger than those of *E. balteatus* and thus may require more aphid prey for development. In a laboratory study, Sharma and Bhalla (1991) showed that *Scaeva pyrastris*, a closely related species to *S. selenitica*, larvae consumed more aphids than *E. balteatus*. Additionally, *E. balteatus* is much more abundant than *S. selenitica* (Maibach, Goeldlin de Tiefenau, & Dirickx, 1992). Larger distances between conspecifics might explain the higher activity in *S. selenitica*, particularly during reproduction when the flies are searching for mates.

An interesting area of further research would be to investigate whether consistent behavioural differences are still present in F1 generations reared from the different phenotypes. Furthermore, it would be important to demonstrate this pattern with a wider range of behavioural tests, such as exploration or boldness, preferentially in different contexts, and attempt to link this to genetic differences. A previous study using microsatellites did not detect any genetic differentiation between overwintering strategies of *E. balteatus* (Raymond, Plantegenest, Gauffre, et al., 2013). However, genetic differentiation between migratory traits may not be detected using neutral markers (Liedvogel, Åkesson, & Bensch, 2011). Genomic methods have been used to uncover differences between migratory and nonmigratory populations of insects (e.g. Zhan et al., 2014; Zhu et al., 2009). More recently, Jones et al. (2015) revealed a set of candidate genes that were differentially expressed in moths that showed an increased propensity for migratory flight.

In summary, we have shown that individuals of two hoverfly species consistently differed in activity and stress latency. Furthermore, there were consistent differences in activity between migratory and resident phenotypes of *E. balteatus*. These results provide evidence for the possible role of behavioural differences in influencing the occurrence of partial migration within populations and warrants careful future investigation.

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## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2017.03.015>.

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