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Fuelling flight in a parasitic wasp: which energetic substrate to use?

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Abstract. 1. Flight is an energy-demanding behaviour in insects. In parasitic wasps, strategies of nutrient acquisition and allocation, resulting life-history trade-offs and relationships with foraging strategies and resource availability have received much attention. However, despite the ecological importance of dispersal between host and food patches, and the great impact energy diverted to flight should have on lifetime reproductive success, the eco-physiology of flight in parasitoids is poorly understood.

2. The objective of this study is to (i) identify the energetic resources used to fuel flight, and (ii) relate nutrient type and rate of utilisation to selective pressures in terms of resource availability posed by the environment.

3. Using a flight mill and biochemical assays, we compared flight performance and nutrient dynamics during flight between two reproductive modes of the parasitoid *Venturia canescens* Gravenhorst, which is known to thrive preferentially in contrasted environments (i.e. natural vs. anthropogenic habitat), differing notably in host and food distribution.

4. Biochemical analyses of different nutrient types showed that glycogen is the flight fuel used by this species, yet no significant differences in its dynamics in flight were found between the two reproductive modes.

5. Results suggest that both glycogen quantity and flight performance are related to the diverging ecological conditions experienced by thelytokous and arrhenotokous strains.

Key words. Arrhenotoky, flight mills, Hymenoptera, nutrient dynamics, thelytoky, *Venturia canescens.*

Introduction

Dispersal behaviour is essential for many insects to find potential mates, suitable reproductive substrates, and food sources. The evolution of wings has facilitated insects with the means to disperse more readily over larger distances, but also imposes severe fitness costs. The cost of wings and wing musculature has been documented extensively in wing polymorphic species (Zera & Denno, 1997). The development of wings and wing musculature poses a major energetic cost and flight is one of the most energy-demanding behaviours (Candy *et al*., 1997; Harrison & Roberts, 2000). During insect flight, metabolic rates can show a 50–100-fold increase (Beenakkers *et al*., 1984). Consequently, flight is a key component in determining the energy budget available to insects, with implications for resource allocation, life-history strategies as well as population dynamics (Guerra, 2011).

Studies on the eco-physiology of flight in insects reveal a high interspecific variability in energetic substrates (i.e. lipids, carbohydrates, or proteins) used to fuel flight. Some insect species appear to fuel flight with carbohydrates only, with both carbohydrates and lipids, or primarily proteins or amino acids, such as proline (Beenakkers *et al*., 1984). In Lepidoptera, Hemiptera, and Orthoptera, lipids are the main source of energy for prolonged periods of flight (Beenakkers *et al*., 1984; Ziegler & Schultz, 1986; Zera *et al*., 1999;

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Canavoso *et al*., 2003; Arrese & Soulages, 2010). In the Colorado potato beetle, *Leptinotarsa decemlineata* Say, proline represents the main energy source to power flight (Weeda, 1979). This corresponds to an indirect use of lipids, as the synthesis of proline requires fatty acid mobilisation (Gäde & Auerswald, 2002). Long distance flyers, such as the locust *Locusta migratoria* Linnaeus, primarily use trehalose to fuel flight, but switch to lipids as the main energy source when trehalose concentrations drop below a certain threshold (van der Horst, 1980). Alternatively, carbohydrates can be solely used to fuel insect flight, as has been observed in the short-distance flying cockroach *Periplaneta americana* Linnaeus (Elliott *et al*., 1984) and bees (Vogt *et al*., 2000; Suarez *et al*., 2005). Ecological conditions, in terms of food, mate, or oviposition substrate location may largely determine flight time, leading to species-specific adaptations in nutrient type allocation to sustain dispersal behaviour.

Hymenopteran parasitoid wasps are fascinating models to study foraging strategies and dispersal behaviour in relation to energy acquisition, allocation, and life-history trade-offs (Jervis & Kidd, 1986; Casas *et al*., 2005; Bernstein & Jervis, 2008; Strand & Casas, 2008; Jervis *et al*., 2008; Pelosse *et al*., 2011). A female's reproductive success is determined by her relative investment of energy and time between current and future reproduction in a given environment (Jervis & Kidd, 1986; Richard & Casas, 2009). Adult females forage for hosts, in which successful ovipositions provide 'immediate' gains in terms of a mother's fitness (Bernstein & Jervis, 2008). However, in most parasitoid species, females also forage for sugary sources that enhance 'future' fitness rewards. Indeed, even if feeding may coincide with reduced oviposition opportunities (Sirot & Bernstein, 1996), it allows for an increase in lifespan and potentially oogenesis. In host-feeding parasitoids, the hosts themselves can further provide a potential food source (Jervis & Kidd, 1986). However, many species solely feed on sugary food sources (i.e. nectar and honeydew) that are typically spatially separated from hosts (Jervis & Kidd, 1986). Here an additional constraint arises regarding optimal time allocation, as time can be allocated either into searching for food or hosts.

Theoretical and empirical studies suggest that the relative investment in host versus food foraging depends both on the probability of finding food and on a forager's energetic state (Sirot & Bernstein, 1996; Lucchetta *et al*., 2007; Bernstein & Jervis, 2008). In these studies, energetic state was considered as a single metric. However, in insects, and particularly parasitoids, nutrient dynamics is a multidimensional process, with several interacting nutrient types not necessarily serving the same purposes and being differentially acquired and used throughout the insect's lifetime (Casas *et al*., 2005; Strand & Casas, 2008; Jervis *et al*., 2008).

Despite the ecological importance of flight in terms of dispersal between host and food patches, and its physiological implications for energy dynamics, the eco-physiology of flight has rarely been studied in parasitic wasps. Moreover, nutrient dynamics during flight through precise quantifications of various nutrient types (i.e. proteins, carbohydrates, and lipids) have never been performed in parasitoids and understanding which nutrients fuel flight and how energy dynamics affect life-history traits is a primordial question to ask.

This study aims at improving our understanding of the eco-physiology of flight in Hymenoptera, particularly parasitoids, by assessing which energetic substrate is used during flight and linking energy dynamics to environmental conditions. The species under study, *Venturia canescens* G. (Hymenoptera: Ichneumonidae), is a synovigenic parasitoid (i.e. able to mature eggs as an adult) that lacks lipogenesis (like many other parasitoid species, Visser & Ellers, 2008 for a review; Moiroux *et al*., 2010; Visser *et al*., 2010) and feeds on sugary substances only (Casas *et al*., 2003; Desouhant *et al*., 2010). It exhibits two strains known to thrive in contrasting environments and that resort to different reproductive modes: a sexual or arrhenotokous strain, and an asexual or thelytokous strain (Beukeboom *et al*., 1999; Schneider *et al*., 2002). Arrhenotokous wasps are found exclusively in natural habitats where host density is low, implying the necessity for frequent flights between host patches. In contrast, thelytokous wasps primarily live in buildings where hosts are relatively abundant (see table 1 of Pelosse *et al*., 2007). Food availability also differs between environments, in which food is more readily available in the field, opposed to a lack of suitable food sources within buildings. Previous comparisons between the two reproductive modes showed that resource allocation strategies correspond to characteristics of the preferred habitat for each strain, with arrhenotokous wasps favouring maintenance-related functions and thelytokous wasps reproduction-related functions (Pelosse *et al*., 2007, 2010).

Using a flight mill system combined with biochemical assays and characterisation of life-history traits, we compared flight performance and energy dynamics in arrhenotokous and thelytokous wasps to identify the energetic substrates used to fuel flight in parasitoids. We expect a higher flight performance and larger energetic compartment dedicated to flight in arrhenotokous wasps, as lifetime reproductive success relies more heavily on flight performance between host patches. Scarcity of sugar sources in the preferred habitat of thelytokous wasps leads to the expectation that flight may be complementarily fuelled with lipids.

Materials and methods

Insect culture

Venturia canescens is a koinobiont endoparasitoid of lepidopteran larvae, mainly Pyralidae (Salt, 1976). This parasitoid is solitary (i.e. only one offspring can develop per host). Females of both reproductive modes emerge with mature and immature eggs (Trudeau & Gordon, 1989; Harvey *et al*., 2001) that mature during adult lifetime (i.e. synovigeny *sensu* Jervis *et al*., 2001). *Venturia canescens* produces small, yolk-deficient hydropic eggs (Le Ralec, 1995) and solely feeds on sugary food sources under natural conditions (Casas *et al*., 2003; Desouhant *et al*., 2005, 2010). Host larvae are concealed within the substrate (fruits or stored products) and localised via a release of kairomones (mandibular gland secretions released when eating) (Corbet, 1971; Castelo *et al*., 2003).

The wasp culture was originally established from adults collected in the field near Valence (44°98′N, 4°93′E, INRA, Gotheron), southern France, on several occasions during the summer of 2008. The host, *Ephestia kuehniella* Zeller, was reared in the laboratory on organic wheat semolina medium (eggs from Biotop mass rearing facility located in Antibes, France). Parasitoid development and experiments took place between February and May 2010 in a controlled environment (24 ± 1 °C; 80 ± 5% RH; LD regime 13 h : 11 h).

Experimental design

To compare nutrient dynamics during flight, females of both reproductive modes were attached to a flight mill for different time periods (named t_{mill} henceforward). For each reproductive mode, freshly emerged, unfed females were randomly assigned to two groups: a control group, in which female nutrient dynamics were determined in the absence of flight (*control* group, $n = 95$), and a treatment group, in which females were tested using the flight mill (*flight* group, $n = 129$). t_{mill} can be either 10 [$n_{thely} = 10$, $n_{arrh} = 8$ with $n_{thely}(n_{arrh})$ number of thelytokous (arrhenotokous) females], 30 ($n_{thely} = 21$, $n_{arrh} = 24$), 60 ($n_{thely} = 18$, $n_{arrh} = 27$), or 120–180 min ($n_{thely} = 11$, $n_{arrh} = 10$). The variance in the longest t_{mill} is explained by the fact that the first observations revealed that 120 min are long enough to reach the maximum time a female is willing to fly under our experimental conditions. From this observation onward we restricted the longest time to 120 min. After flying each female was frozen at −20 °C, after which nutrient levels were quantified using biochemical assays (see below; Foray *et al*., 2012). Each day, two females of each reproductive mode randomly chosen among emerging wasps were assigned to the *control* group. These animals were frozen at a time randomly chosen between 10.00 and 18.00 hours (i.e. the time the flight experiments were performed) to establish baseline nutrient dynamics without flight. The size and weight of flown and a random sample of control females were measured as described below.

Flight mills

A computer-linked flight mill system was used to record wasp flight characteristics, similar to the ones described for moths (Schumacher *et al*., 1997; Hughes & Dorn, 2002; Hughes *et al*., 2004) and parasitoids (*Cotesia glomerata* Linnaeus: Wanner *et al*., 2006; *V. canescens*: Liu *et al*., 2009; Lukáš *et al*., 2010; *Ibalia leucospoides* Hockenwarth: Fischbein *et al*., 2011). Females of both reproductive modes were individually tested on the flight mill on the day of emergence. All females (including those of the control group) were anaesthetised with CO_2 for a maximum of 1 min. Then, the back of the thorax was fixed with cyanoacrylate glue to an insect pin with the upper half of the pin removed. The pin was attached to a plastic flight arm with a radius of 10.5 cm by inserting it vertically through the plastic. A 3-cm long pin, which was also inserted vertically through the flight arm, served as an axis. The pin was placed between two magnets and held upright by the magnetic field. Friction was minimised, because only the tip of the needle was in contact with the magnet. Rotations of the flight mill were monitored by an infrared emitter/receiver through interrupting the beam at each revolution of the flight arm. In one complete revolution of the flight arm, wasps flew 65.97 cm. A single revolution never lasted more than 10 s (S. Besnard, pers. obs.), and we therefore considered that flight was interrupted when flight activity was ceased for more than 10 s. The number of revolutions and cumulative time in flight were recorded.

Sixteen wasps were tested each day using four different mill units. The sequence of four t_{mill} treatments was randomised every day and for each t_{mill} treatment (10, 30, 60, or 120–180 min), two females of each reproductive mode were randomly assigned to a flight mill. Flight was stimulated by the presence of standardised and highly attractive host patches nearby each mill unit.

Weight, egg load, wasp size, and wing loading

Body mass was determined before flight (balance Voyager® pro, ± 0.1 mg). Frozen females (i.e. females of *flight* and *control* treatments) were washed in buffer solution (100 mM KH_2PO_4, 1 mM DTT and 1 mM EDTA, pH = 7.4) for 10 s to remove potential nutrient residues from their bodies. The reproductive system (ovaries + oviducts) was then dissected in 10 µl of buffer solution under a coverslip, and the left hind tibia and left wing removed. Pictures of eggs, wings, and tibiae were captured with a stereomicroscope (Zeiss stemi 2000-C, magnification: × 2.5; Carl Zeiss S.A.S., France) coupled with a Motic® M100 Digital Camera (Motic China Group Co., Ltd, China). The cover slip was then carefully removed and whole egg complement recovered and reloaded with the remaining wasp body in a 2 ml Eppendorf vial by washing the coverslip with 50 µl buffer solution. To minimise enzymatic protein digestion, dissections were performed on ice trays, never exceeded more than 4 min, and dissected wasps were deep-frozen as soon as the procedure was completed. Mature egg load, tibia length, and wing surface were measured from the pictures using the software Motic Images Plus® 2.0 ML (Motic China Group Co., Ltd). We considered eggs to be mature when they were present in the oviducts or calyces. Hind tibia length was used as an index of adult body size because it is positively correlated with dry body mass in *V. canescens* (Harvey & Vet, 1997; Roberts & Schmidt, 2004). Wing loading, defined as the ratio between body mass and wing surface, was measured for females of the *flight* group, as it likely contributes to dispersal ability (Berwaerts *et al*., 2002).

Nutrient assays

To estimate nutrient dynamics of wasps belonging to *flight* and *control* groups, we used a colorimetric method (modified from van Handel, 1985a,b; Giron *et al*., 2002) and applied to *V. canescens* (Pelosse *et al*., 2011; Foray *et al*., 2012)). Assays were performed in 96-well microplates, using a protocol comparable to the one described in Foray *et al*. (2012). Such biochemical assays allow for quantification of different nutrient classes (i.e. carbohydrates, proteins, and lipids) and even

distinguish between circulating sugars in the form of trehalose (glucose–glucose dimer) in the haemolymph and stored carbohydrates present in the form of glycogen (glucose polymer) in the fat body. Assays were performed individually on entire wasps (body + hind tibia + isolated reproductive system).

Data analysis

Life-history traits. ANOVAS were used to investigate the effect of reproductive mode and treatment group (*flight* vs. *control*) on female size and weight. An ANCOVA was used to investigate whether wing loading of females in the *flight* group differed according to their reproductive mode and size. A generalised linear model (log link, Poisson family, and correcting for overdispersion) was used to test for the effect of reproductive mode and treatment group on mature egg load. In these four analyses, two-way interactions were added to the null models.

Nutrient use. Square root transformations were applied to nutrient content to obtain normally distributed data. Then, to remove any effect of animal size from the assays, residuals of the linear model regressing nutrient content against size were used as the response variable in the models described below (hereafter called 'nutrient content residuals').

On the flight mill ($t_{mill} = 10$, 30, 60, or 120–180 min), wasps were free both to stop (because of exhaustion or lack of motivation) and to start flying. We discriminated the time spent resting (t_{rest}) and flying (t_{fly}). Time flying was calculated as $t_{fly} = t_{mill} - t_{rest}$.

To investigate the changing dynamics of nutrient contents, with an accelerating decline after some threshold time flying, generalised additive model (GAM) and threshold model (Ulm, 1989) were used. These revealed neither non-linearities nor threshold value in our data. Hence, linear models were used to test for the effect of reproductive mode, t_{fly} and their interaction, on the four 'nutrient contents residuals' (proteins, lipids, free carbohydrates, and glycogen). Wing loading was added as the first explanatory variable in the model to remove any potential effect its correlation with nutrient contents could have.

By assuming that rates of nutrient consumption by females resting on the flight mill equal those of control females (estimated for thelytokous and arrhenotokous wasps by the coefficients of the full ANCOVA model regressing 'nutrient content residuals' of the *control* group wasps against time × reproductive mode), one could correct the nutrient contents determined at t_{mill} for the nutrients consumed during the t_{rest} minutes of resting. Such correction does not qualitatively affect the results and is not presented here.

Flight performance. Flight performances of thelytokous and arrhenotokous females were compared with a generalised linear model assuming a Poisson distribution of errors (log link and correcting for overdispersion). The response variable was the total number of revolutions performed, and the explanatory variables were t_{fly}, the reproductive mode and their interaction. Wing loading and female size were also added as first explanatory variables as these are known to affect flight performance (Lukáš *et al.*, 2010). Finally, the t_{fly}^2 term and its interaction with reproductive mode were added to the model to account for possible non-linearity in the effect of time spent flying on the distance flown.

For all analyses a backwards procedure of variable elimination through model comparisons with ANOVAS was used to identify a simplified model in which all explanatory variables would significantly affect the response variable. All analyses and graphics were computed using the R free statistical software (R Development Core Team, 2011).

Results

Life-history traits

Mean weight and egg load of females did not differ significantly between *flight* and *control* groups (weight: $F_{1,186} = 0.49$, $P = 0.58$ and egg load: $F_{1,221} = 1.24$, $P = 0.267$, Fig. 1), but difference in mean hind tibia length was marginally significant ($F_{1,221} = 3.51$, $P = 0.06$), wasps from the flying group being slightly larger (Fig. 1a). This may not impact the results as nutrient contents are corrected by size. Arrhenotokous females were smaller ($F_{1,222} = 12.93$, $P = 4\ 10^{-4}$, Fig. 1a) and slightly heavier ($F_{1,187} = 4.546$, $P = 0.034$, Fig. 1b) than thelytokous ones. The mature egg load of arrhenotokous females was lower ($F_{1,222} = 869.16$, $P < 10^{-16}$, Fig. 1c). For both thelytokous and arrhenotokous females, wind load increased with size ($F_{1,126} = 58.44$, $P = 4.65 \times 10^{-12}$) and arrhenotokous mean wing loading was higher ($F_{1,126} = 24.18$, $P = 2.7 \times 10^{-6}$ corrected for size; Fig. 1d).

Nutrient use

The analyses of nutrient consumption while flying revealed that the protein, lipids and free carbohydrate levels depended neither on the reproductive mode (proteins: $F_{1,126} = 0.77$, $P = 0.38$; lipids: $F_{1,126} = 1.84$, $P = 0.18$; free carbohydrates: $F_{1,126} = 0.9$, $P = 0.34$), nor on the time spent flying (proteins: $F_{1,127} = 0.61$, $P = 0.44$; lipids: $F_{1,127} = 0.03$, $P = 0.85$; free carbohydrates: $F_{1,127} = 0.29$, $P = 0.59$). Glycogen was the only nutrient to significantly decrease ($F_{1,126} = 5.64$, $P = 0.019$) during flight. Arrhenotokous females globally had a higher glycogen content ($F_{1,126} = 17.59$, $P = 5.15 \times 10^{-5}$). Results further suggest that both thelytokous and arrhenotokous females consumed glycogen at a similar rate while flying (interaction between reproductive mode and t_{fly} $F_{1,125} = 1.22$, $P = 0.27$; Fig. 2).

Flight performance

After removing the effect of size and wing loading, the number of revolutions performed by arrhenotokous females increased faster with time flying than thelytokous females

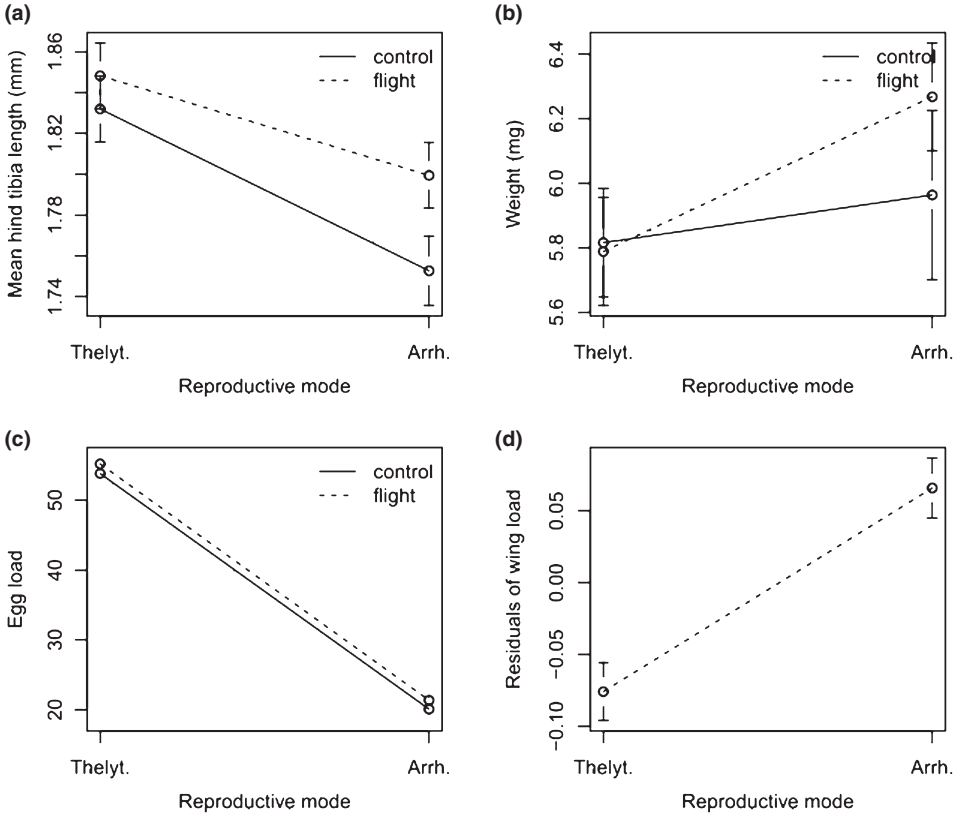

Fig. 1. Life-history traits of thelytokous (Thelyt.) and arrhenotokous (Arrh.) females from the *control* (solid line) and *flight* group (dashed line). (a) mean hind tibia length ± SE (mm), (b) weight ± SE (mg), (c) egg load ± SE, and (d) wing loading corrected for size ± SE (mg.(mm^2)−1). Wing loading was measured only for females belonging to the *flight* group. Residuals were obtained by linear regression of wing loading against size.

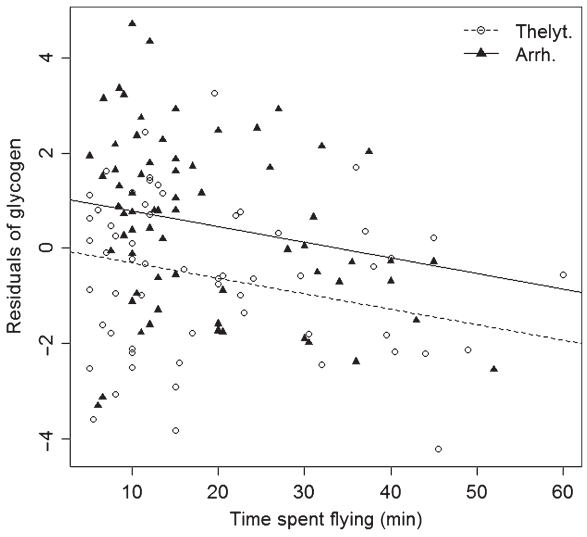

Fig. 2. Residuals of glycogen content in µg (corrected by size and wing loading) of thelytokous (circle, dashed line) and arrhenotokous females (filled triangle, solid line) as a function of the time spent flying (in min). Time spent resting was excluded from the data (see text).

(interaction between reproductive mode and t_{fly} $F_{1,123} = 5.42$, $P = 0.022$, Fig. 3), which means that arrhenotokous females flew faster (average speeds of thelytokous and arrhenotokous females while flying were 0.127 ± 0.0041 and 0.156 ± 0.0057 m s^{-1} (mean ± SE), respectively). For both thelytokous and arrhenotokous females, the speed was a decelerating function of time flying (t^2_{fly}, $F_{1,123} = 111.76$, $P < 10^{-16}$, Fig. 3).

Discussion

Our study identifies carbohydrates, and in particular glycogen, as the fuel used during flight in the parasitoid *V. canescens*, and contributes to clarify the question of nutrient use within Hymenoptera. Our results also suggest that arrhenotokous wasps, which fly more frequently between host and food patches than thelytokous ones in their preferred habitat, have a higher flight potential than the latter. Indeed, emerging arrhenotokous females possess a higher glycogen load and fly faster. Surprisingly, the higher flying performance of arrhenotokous females does not seem to be positively correlated with a higher nutrient consumption rate.

In *V. canescens*, glycogen is the only energetic compartment that decreases during flight. Our results show that females

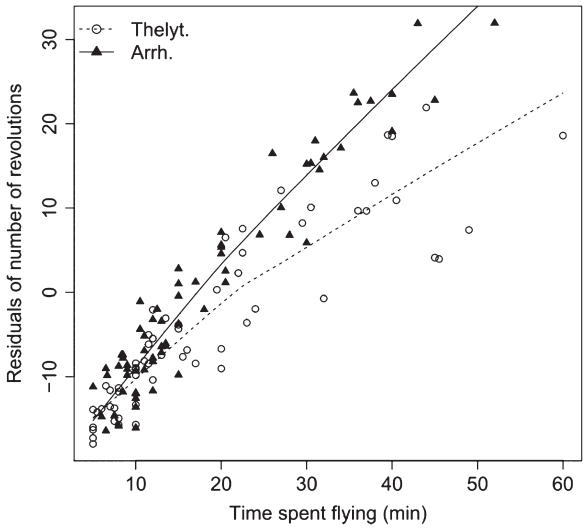

Fig. 3. Residuals of number of revolutions (corrected by size and wing loading) performed by thelytokous (circles, dashed line) and arrhenotokous (filled triangles, solid line) females as a function of the time spent flying in the flight mills (in min).

consume about 50% of their glycogen content in 1 h of flight (predictions from statistical models for thelytokous: from 17.7 to 6.37 μg glycogen mm^{-1} of tibia length and arrhenotokous: from 25.7 to 14.3 μg glycogen mm^{-1}). These quantities may correspond to the consumption of more than half the available glycogen (Pelosse *et al*., 2010). This decrease is more than seven times higher than that of control females (results not shown). However, their glucose, lipid, and protein contents remain constant. To be used by flight muscles, glycogen has to be cleaved to produce glucose in haemolymph and flight muscles (van der Horst, 2003). A decrease of glucose concentration in the haemolymph induces glycogenolysis (Ziegler & Schultz, 1986), which may explain why our glucose measurements were constant. Free sugar might decrease when glycogen is running out. Determining whether animals undergoing the strong decline in glycogen have also started to use other nutrients, in particular sugars, would have required to use isotope signature to follow each nutrient (as in Giron & Casas, 2003). In the wild, opportunities to feed may, under positive balance of intakes (feeding) and outputs (energetic demands) allow carbohydrates to be stored as glycogen for subsequent use (Olson *et al*., 2000; O'Brien & Suarez, 2001; Pelosse *et al*., 2010). Intense muscular performances, such as flight, requires mobilisation of energy stored in the fat body (Gäde *et al*., 2006). The composition of the fat body is highly stable among insects (mainly triglycerides, and to a lesser extent glycogen; Arrese & Soulages, 2010). However, the way it is used to fuel flight seems to be highly variable between insect orders (see Introduction). For instance in hymenopterans, orchid bees (Suarez *et al*., 2005) and ants (Vogt *et al*., 2000) have been shown to use carbohydrates. Uncertainties remained about wasps, notably parasitic wasps, as no direct measures of nutrient dynamics have been done. In *Cotesia glomerata* wasps, Wanner *et al*. (2006) suggest that parasitic wasps use carbohydrate to fuel flight as adult feeding on floral nectar increases flight capacity. In contrast to these findings, Fischbein *et al*. (2011) have shown that flight parameters in *Ibalia leucospoides* were not affected by previous access to sugary food sources. In *Asobara tabida* Nees, a decrease in fat reserves of females captured at longer distances from a release site was observed, suggesting use of lipids to fuel flight (Ellers *et al*., 1998). Nevertheless, a concomitant use of glycogen for flight cannot be excluded in this species, as it was not quantified.

Several species of Hemiptera (Gäde *et al*., 1996; Canavoso *et al*., 2003), Lepidoptera and Orthoptera use carbohydrates to initiate flight only, and then supplement it with lipids. We cannot rule out the fact that under certain conditions, or during longer flights, *V. canescens* uses lipids to supplement carbohydrates. Foraging strategies (i.e. the length and frequency of flights) could account for the variability in the type of nutrient used to fuel flight (Niitepõld *et al*., 2009). The spatial distribution of resources may determine the quantity of energy that has to be stored and the speed of mobilisation of nutrients required to reach each resource patch (Mentel *et al*., 2003). Indeed, triglycerides provide a higher caloric content per unit of weight than glycogen, but glycogen is more rapidly mobilised than lipids (Stryer, 1981). Locusts that are susceptible to undergoing long periods of starvation or to perform long distance flights fuel them with lipids (van der Horst & Rodenburg, 2010). Conversely, bee or ant foragers that perform short and frequent flights, returning to their nests several times a day, or parasitoids searching for hosts or food may require the use of rapidly accessible nutrients. In the wild, *V. canescens* (mainly arrhenotokous ones) frequently feeds on sugar-rich food sources (Casas *et al*., 2003; Desouhant *et al*., 2010), which are widely available as floral nectar and leaf or fruit exudates (Desouhant *et al*., 2005). These frequent sugary inputs may allow glycogenesis (Olson *et al*., 2000; O'Brien & Suarez, 2001; Pelosse *et al*., 2010), a phenomenon probably impeded in our experiment due to the absence of food. Study of navigation rules used by *V. canescens* searching for hosts in the wild (Desouhant *et al*., 2003) suggests that the mean duration of flight in our experiment is ecologically realistic. Hence, ecological conditions in the field may not act as selective pressure for the supplementation of carbohydrate by lipids to fuel flights.

Data obtained from flight mills allow only for an estimation of flight capacity (Riley *et al*. 1997; Bruzzone *et al*., 2009; Taylor *et al*., 2010). Nevertheless, recent comparative studies report consistent results between activity patterns in the laboratory and in the field (Taylor *et al*., 2010; Niitepõld *et al*., 2011). The low variability in weight, size, or wing loading between thelytokous and arrhenotokous females allows comparison between reproductive modes as both thelytokous and arrhenotokous females may be similarly impacted by friction and suspension.

Our results suggest that without feeding, arrhenotokous females have a higher flying potential than their thelytokous counterparts. First, at emergence they have a higher energetic compartment dedicated to flight (glycogen), confirming previous results (Pelosse *et al*., 2007, 2010). Second, they are faster flyers, similar to findings of Lukáš *et al.* (2010). Finally, they

have a higher wing loading, a morphological feature positively correlated to flight speed in Lepidoptera and Hymenoptera (Berwaerts *et al*., 2002; Fischbein *et al*., 2011). If the observed difference in velocity was due to a difference in wing beat amplitude or frequency, one would have expected a higher glycogen consumption rate by arrhenotokous females in comparison with thelytokous ones. Surprisingly, no significant difference in glycogen consumption rate was found between the two reproductive modes, which suggest that arrhenotokous females are flyers that are more efficient. Owing to the high variability of the data, we may also miss statistical power. A comparison of wing beat frequency or oxidative capacities of thelytokous and arrhenotokous females would help clarify whether the latter are more efficient while flying or whether their increased speed is correlated with higher energy expenditure. In any case, the similar basal metabolic rate of the two strains (Foray, 2011) suggests that potential differences of energy expenditure should be of small amplitude. Consequently, the higher velocity of arrhenotokous females, together with their higher initial glycogen content may be advantageous after emergence in the field where frequent flights are needed to find hosts and food under time constraint, and should decrease their risk of predation. While 1 h of flight might be too low to impact egg load of flying females (similar egg load between flight and control females, Fig. 1c), the lower egg load of arrhenotokous females (this study; Pelosse *et al*., 2007) suggests that they could pay their higher performance in flight in terms of fecundity. *Venturia canescens* produce small eggs with little yolk. Whether and how flight impacts other metabolic functions (i.e. reproduction or maintenance) may depend on which nutrient each of them use as fuel and in what quantity. Contrasting our results with the energetics of winged vs. wingless parasitoid species producing yolk-rich eggs would constitute a step further toward our understanding of trade-offs between flight and other metabolic functions (Harvey *et al*., 2012).

In spite of the lack of population replicates, our conclusion converges on those of previous studies conducted with several populations of *V. canescens*. These studies about thermal tolerance (Amat *et al*., 2006; Foray *et al*., 2011), learning ability (Thiel *et al*., 2006; Froissart *et al*., 2012), and patch exploitation strategy (Amat *et al*., 2009) converge to suggest a better adaptation of arrhenotokous females to the ecological conditions encountered in the wild. This could partly explain their regional coexistence with thelytokous counterparts, despite the costs entailing male production. It may allow arrhenotokous females to exploit resources earlier or more thoroughly, and/or reduce the overlap of their ecological niche with the one of thelytokous females.

In addition to the aforementioned resource distribution, fuel use in insect flight could be related to foraging activities via the diet of foraging animals. In Lepidoptera, fuel use has been shown to vary according to diet (O'Brien, 1999). On the contrary, in Hemiptera, enzymatic pathways and lipid utilisation seem to be independent of food regime (herbivorous, predator, or haematophagous) (Gäde *et al*., 2006). Despite the scarcity of sugary food sources in buildings where thelytokous females thrive preferentially, these did not complement glycogen use with lipids. Further studies would help to clarify whether when getting older or under longer flights, thelytokous females resort to lipids. This absence of the use of lipids can also be due to the absence of lipogenesis in *V. canescens* (Casas *et al*., 2003). More generally, parasitoids are relevant biological models, which could help clarify the link between diet and the energetics of flight. Indeed, as lipogenesis is absent in a large number of parasitoid species (Visser & Ellers, 2008 for a review; Moiroux *et al*., 2010; Visser *et al*., 2010) adults can rely mainly only on the lipid resources acquired as larvae. Parasitoids show contrasting feeding strategies: host vs. non-host-feeders (Jervis & Kidd, 1986). While the former can provide few lipids, the latter is mainly composed of carbohydrates (Jervis & Kidd, 1986; Giron *et al*., 2002; Strand & Casas, 2008; Zhang *et al*., 2011). To our knowledge, no study has assessed whether host-feeding parasitoids fuel flight with lipids.

To our knowledge, our study is the first to identify, via biochemical measurements, the nutrients consumed during flight in a parasitic wasp. Such measures may prove useful when investigating the potential impact of flight on the expression of the trade-off between dispersal capability and reproduction in many insect taxa (Guerra, 2011). Parasitoid wasps are relevant biological models to test whether and how the ecological context influences which nutrients are used to fly. Indeed, parasitoids present a high diversity in life-history traits and habitats, which may impact their energetic budget and the way physiological trade-offs are solved. For instance, *Leptopilina boulardi* Barbotin in desert areas differ from non-desert females in their ability to synthesise lipids (Moiroux *et al*., 2010). This species offers the opportunity to test whether flight can be fuelled by lipids in parasitoids experiencing extreme ecological conditions (in terms of host, food, or water availability).

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Contribution of authors:

Conceived and designed the experiments: IA, CB, ED. Performed the experiments: SB. Analysed the data: IA, SB, ED. Wrote the paper: IA, ED. Provided significant help at the final stages of the article: VF, PP, CB.

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