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RESEARCH ARTICLE

Flight energetics, caste dimorphism and scaling properties in the bumblebee, *Bombus impatiens*

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ABSTRACT

Animal size affects the energetics of locomotion. Using female caste dimorphism in bumblebees, we assessed how body mass impacts morphological and physiological traits linked with flight. The allometric relationships obtained for wing surface area, wingbeat frequency, and flight and resting metabolic rates of workers could predict the trait values of queens that were more than fourfold larger. Flight success of queens decreased over time in part because of a large increase in body mass and a decrease in traits linked with flight, namely wingbeat frequency and metabolic rate, and the activity of metabolic enzymes tended to decrease. After taking into account temporal changes, body mass, flight wingbeat frequency and metabolic rate were repeatable. Finally, we found significant family resemblance for all traits measured, indicating that shared genes and/or environmental effects impact phenotypic variation. Together, our results show that the functional association between body morphology and flight physiology is robust, providing further insights into the mechanistic basis of metabolic rate scaling patterns during locomotion in animals.

KEY WORDS: Metabolic rate, Flight, Wingbeat frequency, Muscle, Enzyme, Allometry

INTRODUCTION

The effect of animal size on biological form and function is profound and diverse. The impact of body size on animal locomotion remains central to our understanding of energetics and metabolism (e.g. Alexander, 2005; Dlugosz et al., 2013; White et al., 2016; Dick and Clemente, 2017; Halsey and White, 2017; Hirt et al., 2017). Strategies used to investigate these effects range from interspecific macroevolutionary studies (see citations above), intraspecific studies exploiting interindividual variation (e.g. Skandalis and Darveau, 2012; Chappell et al., 2013; Huang et al., 2013; Darveau et al., 2014) or ontogenetic effects (e.g. McHenry and Lauder, 2006; Kirkton et al., 2011). Experimental manipulation of size and body proportion has also been used, such as allometric engineering (Sinervo and Huey, 1990) or using artificial selection (Frankino et al., 2005). Each approach has its advantages and drawbacks, with a common goal of characterizing scaling patterns, drawing a mechanistic explanation from the observed relationships and testing predictions from the hypothesized mechanisms.

Studies performed on insects have proven useful in addressing the mechanistic basis of body size effects on metabolic rate and its underlying physiological traits. Interspecific studies exploiting a range of closely related species varying widely in body mass have addressed the potential constraints of the tracheal system on oxygen delivery (Kaiser et al., 2007), the physiological and biochemical determinants of flight metabolic rate (Casey et al., 1985; Darveau et al., 2005a,b; Rodriguez et al., 2015), and the impact of animal size on the biomechanics of flight (Casey et al., 1992; Dillon and Dudley, 2004). Interindividual variation has been useful for studying changes in respiratory properties during instar transitions in hemimetabolous insects (Greenlee et al., 2009; Snelling et al., 2011, 2012). The large body size range of some holometabolous insects has enabled investigation of the effects of body mass on morphological and cellular traits associated with flight metabolic rate (Skandalis and Darveau, 2012; Darveau et al., 2014). Predictions based on patterns observed among individuals can also be used to test the impact of sexual dimorphism within species (Darveau et al., 2014). However, few studies to date have used experimental manipulations of size to test predictions from physiological scaling relationships. The ability to generate larger or smaller individuals of a species would enable the predicted impact on their function to be tested, and ultimately bridge the gap between interspecific and intraspecific scaling studies.

Insect body size affects flight kinematics, which in turn drives variation in the metabolic rate of species or individuals. Studies comparing various species of several insect groups have shown that species body mass impacts wing proportion and wingbeat frequency (Bartholomew and Casey, 1978; Casey et al., 1985; Byrne et al., 1988; Darveau et al., 2005a). Recent work from our group has shown that such an association is also found among individuals within species (Skandalis and Darveau, 2012; Darveau et al., 2014). The relationship between body mass, wing morphology and kinematics has also been shown to explain flight metabolic rate variation between species (Casey et al., 1985; Darveau et al., 2005a; Rodriguez et al., 2015), but also variation among individuals (Skandalis and Darveau, 2012; Darveau et al., 2014). Muscle function was also shown to be impacted by body mass, probably due to the influence of size on muscle metabolic intensity as reflected by mass-specific metabolic rate. Species with greater mass-specific flight metabolic rate have higher activities of energy metabolism enzymes (Darveau et al., 2005b), and such an association was also found among individuals within a species (Skandalis and Darveau, 2012; Darveau et al., 2014).

The large variation in body mass found within species can be a powerful tool to test hypothesized associations among functional traits that are often derived from interspecific studies. Hymenopterans can be especially useful in this regard, given that caste polymorphism is found in some social species. Bumblebees such as *Bombus impatiens* have a wide distribution in body size among workers (Couvillon et al., 2010); in addition, queens can be much larger than workers and drones (del Castillo and Fairbairn, 2012). Based on previous work investigating determinants of flight energetics between species (Darveau et al., 2005a,b) and among *B. impatiens* individuals (Skandalis and Darveau, 2012; Darveau

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et al., 2014), we hypothesized that large bumblebee queens will have lower wingbeat frequency and mass-specific flight metabolic rate. Additionally, flight muscle metabolic enzyme activity should be lower in queens given their larger size and predicted lower mass-specific metabolic rate. Finally, data generated on queens and offspring workers allowed us to test family resemblance, thereby providing insight into the potential heritability of the traits associated with flight energetics.

MATERIALS AND METHODS
Animals and holding conditions
Thirty colonies of bumblebees (*Bombus impatiens* Cresson 1863) were donated by a commercial supplier (Biobest Canada Ltd, Leamington, ON, Canada), and 27 colonies were obtained from laboratory rearing of queens collected in the wild in the Gatineau (QC, Canada) region. Commercial colonies were kept in the supplier's housing boxes in a room maintained at ~25°C on a 12 h:12 h light:dark photoperiod. Colonies were provided with unlyophilized pollen and sucrose solution (50% v/v) *ad libitum* and acclimated to these laboratory conditions for 1 week after arrival.

Queens collected in the field were identified and transported to our laboratory using 50 ml plastic tubes with a punctured cap, stored on ice in a portable cooler. Upon arrival, queens were weighed using an analytical balance (Excellence XS, Mettler-Toledo, Mississauga, ON, Canada). They were then placed in individual wooden nest boxes equipped with a glass feeder filled with sucrose solution and provided with pollen *ad libitum*. Nest boxes were placed in an environmental chamber set at 30°C with a 12 h:12 h light:dark photoperiod. Workers were counted every day and colonies that reached 15 workers were transferred into the same housing boxes, room and conditions as the commercially obtained colonies described above.

Flight measurements
Flight metabolic rate (MR) and wingbeat frequency (WBF) measurements were performed on each queen and a subset of 15 of her workers. We initiated the study using commercial bumblebee colonies, and observed an apparent decrease in successful flights of queens over the first week of acclimation. We further documented the repeatability and time dependence of flight measurements in the wild-caught queens by performing four series of flight measurements

Table 1. Sources of variation of worker bumblebee morphological and flight performance traits obtained from mixed-effects models

Body mass (g)	Fixed	β (s.e.)	F	P
	Intercept	−0.763 (0.008)	$8833.88_{1,52}$	<0.001
	Population	−0.014 (0.011)	$1.49_{1,768}$	0.222
	Random	σ^2	$\chi^2_{0.5}$	P
	Colony	1.150×10^{-3}	48.94	<0.001
	Residuals	7.606×10^{-3}		
Wing surface area (mm^2)	Fixed	β (s.e.)	F	P
	Intercept	1.987 (0.013)	$23{,}432.02_{1,41}$	<0.001
	Population	0.008 (0.005)	$2.13_{1,353}$	0.145
	Body mass	0.668 (0.016)	$1701.36_{1,353}$	<0.001
	Random	σ^2	$\chi^2_{0.5}$	P
	Colony	2.36×10^{-4}	48.14	<0.001
	Residuals	7.12×10^{-4}		
WSA (mm^2)	Fixed	β (s.e.)	F	P
	Intercept	1.230 (0.015)	$6436.25_{1,41}$	<0.001
	Population	0.007 (0.005)	$2.02_{1,353}$	0.156
	Body mass	0.637 (0.019)	$1070.46_{1,353}$	<0.001
	Random	σ^2	$\chi^2_{0.5}$	
	Colony	1.63×10^{-4}	16.56	
	Residuals	1.07×10^{-3}		
Flight MR (ml CO$_2$ h^{-1})	Fixed	β (s.e.)	F	P
	Intercept	1.742 (0.020)	$7371.60_{1,52}$	<0.001
	Population	−0.010 (0.007)	$2.01_{1,767}$	0.156
	Body mass	0.829 (0.026)	$1035.40_{1,767}$	<0.001
	Random	σ^2	$\chi^2_{0.5}$	P
	Colony	3.35×10^{-4}	19.54	<0.001
	Residuals	4.27×10^{-3}		
RMR (ml CO$_2$ h^{-1})	Fixed	β (s.e.)	F	P
	Intercept	0.178 (0.049)	$13.08_{1,24}$	0.001
	Body mass	0.867 (0.058)	$221.63_{1,169}$	<0.001
	Random	σ^2	$\chi^2_{0.5}$	P
	Colony	2.24×10^{-3}	26.73	<0.001
	Residuals	6.59×10^{-3}		
WBF (Hz)	Fixed	β (s.e.)	F	P
	Intercept	2.172 (0.008)	$68{,}212.59_{1,52}$	<0.001
	Population	−0.011 (0.003)	$11.76_{1,767}$	<0.001
	Body mass	−0.164 (0.010)	$246.60_{1,767}$	<0.001
	Random	σ^2	$\chi^2_{0.5}$	P
	Colony	1.00×10^{-4}	45.60	<0.001
	Residuals	6.79×10^{-4}		

WSA, wing section area; MR, metabolic rate; RMR, resting metabolic rate; WBF, wingbeat frequency. Note: all variables were log$_{10}$-transformed, such that for all variables except body mass the β estimates for the fixed effects represent the relationship log$Y=b$log$X+$log$a$, where X is body mass. The β estimate for population represents the intercept difference for the commercial colonies compared with the wild-caught bumblebees. F-values are shown with numerator and denominator degrees of freedom.

during the progression of the colony development. The first set of measurements was conducted immediately at the site of capture before transport to our laboratory. The respirometry chamber was placed in a temperature-controlled cabinet [PTC-1, Sable Systems International (SSI), Las Vegas, NV, USA] linked to a temperature controller (Pelt-5, SSI) and maintained at 22±2°C. The second set of measurements was conducted 1 week after the first workers had hatched in the laboratory. The third series of measurements was performed after 40 workers were present in the colony, which corresponded with the size of the commercial colonies when measurements were performed. A final set of measurements was obtained 1 week later. All individuals were then dissected and stored at −80°C for morphological and cellular measurements.

Rates of CO_2 production and WBF were measured as described in other studies (Skandalis and Darveau 2012; Darveau et al., 2014; Rodriguez et al., 2015) using a FoxBox flow-through respirometry system (SSI). All flight measurements were performed at room temperature (21–22°C).

Resting metabolic rate

Metabolic rate at rest (RMR) was measured in commercial colonies only. All RMR measurements were taken between 17:00 h and 09:00 h. Bees were transferred from their respective colonies into small microrespirometry chambers (SSI) in a dark room maintained at 25°C. Using a MUX-3 multiplexer and Flowbar-8 multichannel mass flow meter (SSI) coupled to a sub-sampling pump (SS3, SSI), dried air was pushed into the selected chamber at a rate of 60 and 120 ml min^{-1} for workers and queens, respectively. Air leaving the chamber was dried and CO_2 production was measured using a LiCor 7000 differential CO_2/H_2O analyser (Li-Cor Environmental, Lincoln, NE, USA) and recorded with Expedata acquisition software (SSI). Bees were placed in seven chambers and one chamber was left empty and used as a baseline CO_2 measurement. Each bee was measured for 1 h and CO_2 baseline was monitored before and after in each case. We used data from individuals that showed patterns of discontinuous gas exchange, which we used as an indicator of a resting state (Matthews and White, 2011).

Morphological measurements

Upon completion of whole-animal measurements, individuals were anaesthetized using nitrogen and frozen at −80°C. Individual bees were dissected with scissors and parts (head, thorax, abdomen, wings and legs) were weighed to the nearest 0.1 mg. Wings were removed from each individual and pasted on paper. Digital images of the right forewing were taken for each individual using a camera connected to a dissection microscope (Discovery V8, Zeiss, Oberkochen, Germany). Wing surface area was measured using Axio Vision software (Zeiss) in queens and 10 of their workers. Queens sometimes exhibited extensive wing wear, and therefore whole wing measurements were impossible to perform. As wing venation is conserved within Hymenoptera species (Francoy et al., 2009), we measured a wing section that represented on average 18.46±0.04% of the whole surface area. Wing section area (WSA) scaled with whole wing surface area with an exponent close to isometry (b=0.94, r^2=0.949, P<0.001, n=461).

Enzyme activity measurements

The activity of glycogen phosphorylase (GP), trehalase (TR), hexokinase (HK) and phosphoglucoisomerase (PGI) was measured in bumblebee thorax as described in Darveau et al. (2014).

Statistical analysis

Statistical analyses were performed using the software SYSTAT 13.0 (Chicago, IL, USA). In bumblebee workers, interindividual variation is strongly determined by variation in body mass (Skandalis et al., 2012; Darveau et al., 2014). We therefore examined all relationships with body mass using log-transformed data. For workers, we used linear mixed models to estimate the variation explained by the fixed effects colony population (wild versus commercial) and body mass when applicable, as well as the random effect of colony. Interaction between body mass and population was tested and removed from the models as it was not significant in all cases. For fixed effects, the parameters β and standard error, F-statistics and corresponding P-values are reported. The β value reported for the fixed effect body mass represents the exponent value from the relationship $Y=aX^b$. For random effects, the variance parameter σ^2 is reported, and the significance of variance parameters was tested by comparing the log-likelihood of the full model and a reduced model with the colony random effect removed, which follows a χ^2 distribution with 0.5 degrees of freedom ($\chi^2_{0.5}$) (Dominicus et al., 2006; Niemelä and Dingemanse, 2017). We further performed a Pearson's correlation analysis to assess the relationship between residuals of some flight traits (WSA, WBF and flight MR) obtained from the mixed models described above.

To test for differences in enzyme activity between the types of bees used in the study (workers, gynes, spring queens and queens), we used mixed-effects models including the fixed effects colony population (wild versus commercial), body mass and type.

Table 2. Sources of variation of worker bumblebee flight muscle metabolic enzyme activity obtained from mixed-effects models

GP (U g^{-1})	Fixed	β (s.e.)	F	P
	Intercept	0.986 (0.062)	252.47$_{1,44}$	<0.001
	Population	−0.034 (0.019)	3.15$_{1,209}$	0.077
	Body mass	0.042 (0.081)	0.27$_{1,209}$	0.603
	Random	σ^2	$\chi^2_{0.5}$	
	Colony	2.22×10^{-3}	11.69	<0.001
	Residuals	1.04×10^{-2}		
TRE (U g^{-1})	Fixed	β (s.e.)	F	P
	Intercept	1.632 (0.048)	1147.60$_{1,44}$	<0.001
	Population	−0.062 (0.017)	14.07$_{1,209}$	<0.001
	Body mass	0.025 (0.062)	0.17$_{1,209}$	0.685
	Random	σ^2	$\chi^2_{0.5}$	
	Colony	2.05×10^{-3}	23.24	<0.001
	Residuals	5.99×10^{-3}		
HK (U g^{-1})	Fixed	β (s.e.)	F	P
	Intercept	1.867 (0.039)	2297.86$_{1,44}$	<0.001
	Population	−0.215 (0.054)	16.00$_{1,208}$	<0.001
	Body mass	−0.077 (0.051)	2.31$_{1,208}$	0.130
	Origin×body mass	−0.203 (0.069)	8.53$_{1,208}$	0.004
	Random	σ^2	$\chi^2_{0.5}$	
	Colony	4.41×10^{-4}	13.86	<0.001
	Residuals	1.92×10^{-3}		
PGI (U g^{-1})	Fixed	β (s.e.)	F	P
	Intercept	2.741 (0.046)	3490.21$_{1,44}$	<0.001
	Population	−0.033 (0.016)	4.40$_{1,208}$	0.037
	Body mass	0.108 (0.060)	3.26$_{1,208}$	0.073
	Random	σ^2	$\chi^2_{0.5}$	
	Colony	1.76×10^{-3}	22.72	<0.001
	Residuals	5.53×10^{-3}		

Note: all variables were log$_{10}$-transformed, such that for all variables the β estimates for the fixed effects represent the relationship log$Y=b$log$X$+log$a$, where X is body mass. The β estimate for population represents the intercept difference for the commercial colonies, and the interaction between population and body mass represents the difference in slope for commercial colonies.

In order to evaluate the phenotypic variation of body mass and flight performance traits (flight MR, WBF) in queens, we performed mixed-effects models with colony state (and body mass for flight performance traits) as fixed effect, and queen ID as random effect. Colony state refers to measurements performed at four different stages of the colony: before the establishment of the colony, 1 week after the emergence of the first worker, when the colony reached 40 workers and 1 week after this point. Repeatability was calculated from the variance parameters obtained from the mixed-effects models and significance was determined from the $\chi^2_{0.5}$ obtained for the random effect.

Using data on queens and their offspring, family resemblance was evaluated. We first tested whether offspring mean values were related to queen trait values using linear regression. When applicable, we accounted for the effect of body mass or population origin by using residuals according to the results of the mixed-effects models in Tables 1 and 2. We also used the intra-class correlation coefficient to determine whether siblings resemble each other more than individuals of other families. The intra-class correlation coefficient was calculated from the variance parameters obtained from the mixed-effects models and significance obtained from the $\chi^2_{0.5}$ values obtained for the random effect.

RESULTS

Phenotypic variation in workers and queens

For all variables measured in bumblebee workers, we first assessed the effects of population of origin, wild versus commercially obtained bumblebees, using a mixed model including population, and body mass when applicable, as fixed effects, and colony as random effect. Flight WBF was higher in wild colony workers (Table 1 and Fig. 1). Wild colony workers also had higher activity of the enzymes TR, HK, PGI and GP, approaching significance ($P=0.077$) (Table 2 and Fig. 2).

The effect of worker body mass on morphological and energetic parameters is summarized in Table 1. Wing surface area scaled
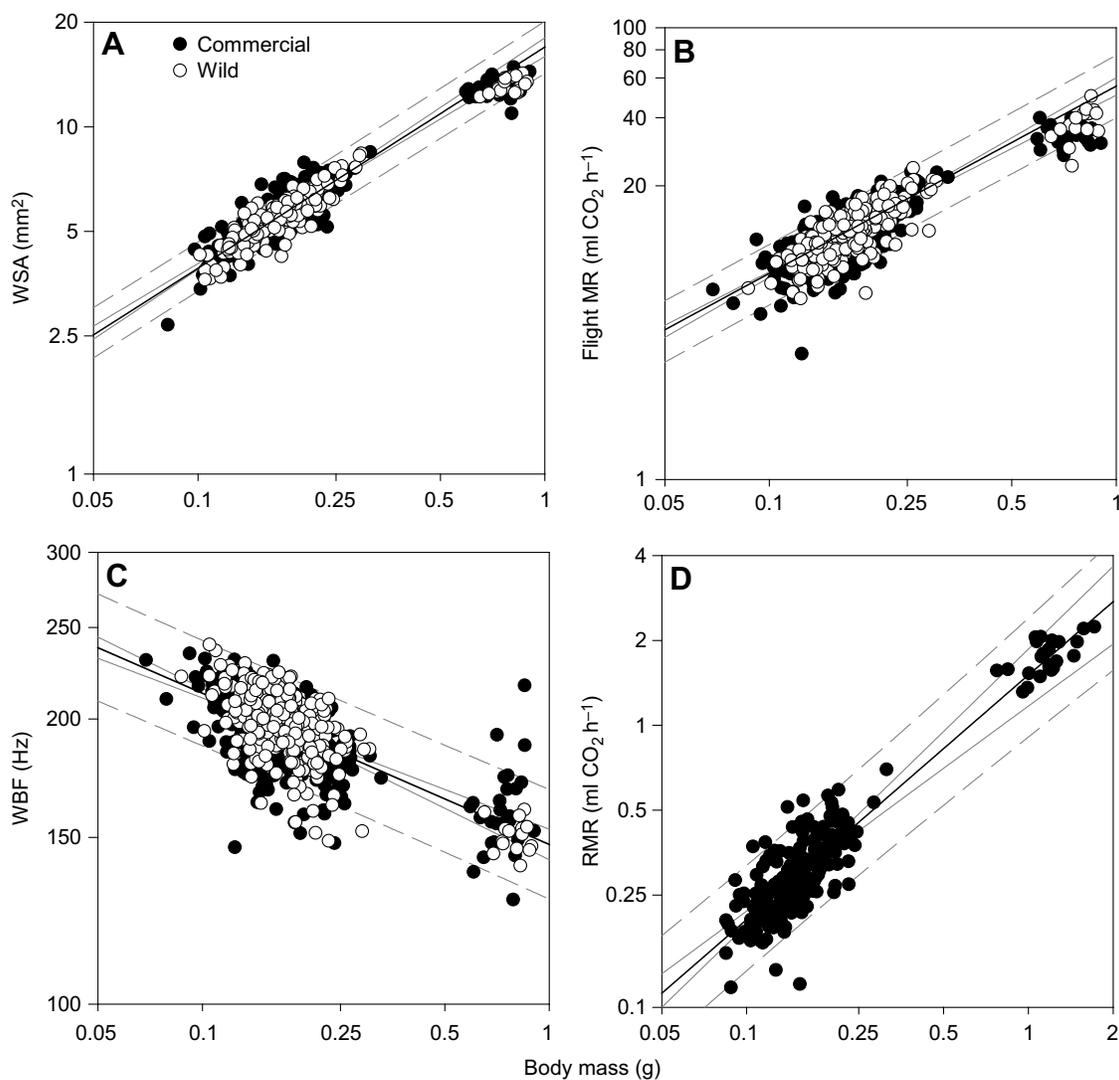
Fig. 1. The effects of body mass on traits of worker and queen bumblebees. Relationship between body mass and (A) wing section area (WSA; workers: $n=397$; queens: $n=42$), (B) flight metabolic rate (flight MR; workers: $n=822$; queens: $n=42$), (C) wing beat frequency (WBF; workers: $n=822$; queens: $n=42$) and (D) resting metabolic rate (RMR; workers: $n=195$; queens: $n=24$) in commercial and wild *Bombus impatiens* workers and larger size queens. Regression lines are for linear regressions performed on workers only and are extended to the axes to locate queens in relation to the predictions from worker relationships. The 95% confidence intervals (solid lines) and prediction intervals (dashed lines) are presented. Additional variables tested using mixed-effect models are presented in Table 1.
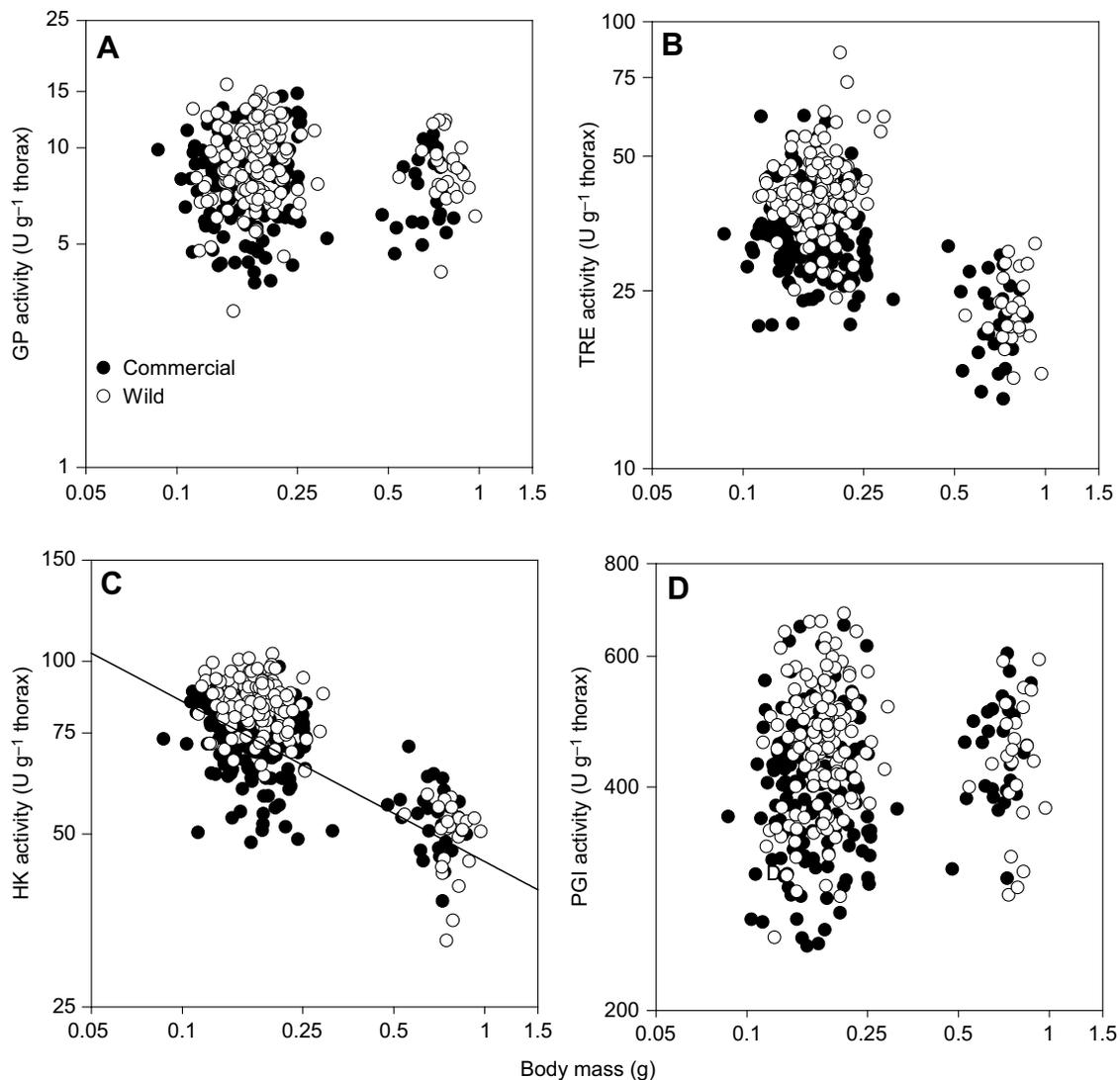
Fig. 2. Flight muscle enzyme activity in worker and queen bumblebees. Relationship between body mass and the activity of (A) glycogen phosphorylase (GP), (B) trehalase (TRE), (C) hexokinase (HK) and (D) phosphoglucoisomerase (PGI) in commercial and wild *B. impatiens* workers (*n*=306) and queens (*n*=52). Sources of variation in enzyme activity of workers obtained from mixed-effects models are reported in Table 2. For HK, regression is shown for commercial workers only using parameters from Table 2, and extended to axes to locate queens in relation to the predictions from the worker relationships.

isometrically with an exponent value of 0.668; WSA selected for estimation of surface area for an individual with a damaged wing scaled with a slightly lower exponent (*b*=0.637). Flight MR and RMR scaled allometrically with worker body mass with exponent values of 0.829 and 0.867, respectively. WBF decreased with increasing body mass with scaling exponent of −0.164. Variation in wing surface area and WBF, after accounting for body mass, was negatively correlated, and flight MR and WBF were positively correlated (Fig. 3).

Measurements performed on queens showed that the wild population had higher flight MR than that of commercial queens (population: $F_{1,40}$=6.87, *P*=0.012; body mass: $F_{1,40}$=2.08, *P*=0.156), and that commercial queens had a higher WBF than wild ones (population: $F_{1,40}$=6.41, *P*=0.015; body mass: $F_{1,40}$=1.92, *P*=0.174). Populations did not differ in WSA (population: $F_{1,40}$=0.65, *P*=0.42; body mass: $F_{1,40}$=8.25, *P*=0.006). Compared with workers, queen values were within the 95% prediction limits obtained from the regressions performed on workers as represented in Fig. 1.

Measurements conducted on wild queens at various time points show that queen phenotype changed over time. Queen mass increased significantly from the time of capture before the establishment of the colony to 1 week after the emergence of the first worker, and further increased by the time the colony was well established with over 40 workers (Fig. 4A). After accounting for body mass, flight MR and WBF increased from the time of capture to the time the colony was just established, and further declined as colony development progressed (Fig. 4C, E). Although queen body mass, flight MR and WBF changed over time, after accounting for temporal changes all traits remained repeatable (Fig. 4B,D,F).

We compared workers with gynes in the laboratory, young queens collected in the spring, and gravid queens with established colonies (Fig. 5). Using mixed models with population of origin, body mass and bee type as fixed effects, analyses revealed that workers had higher GP activity than gravid queens (Fig. 5A; *P*=0.037). The activity of TRE and HK was also the lowest in gravid queens and different from that in workers (Fig. 5B,C; *P*<0.01).
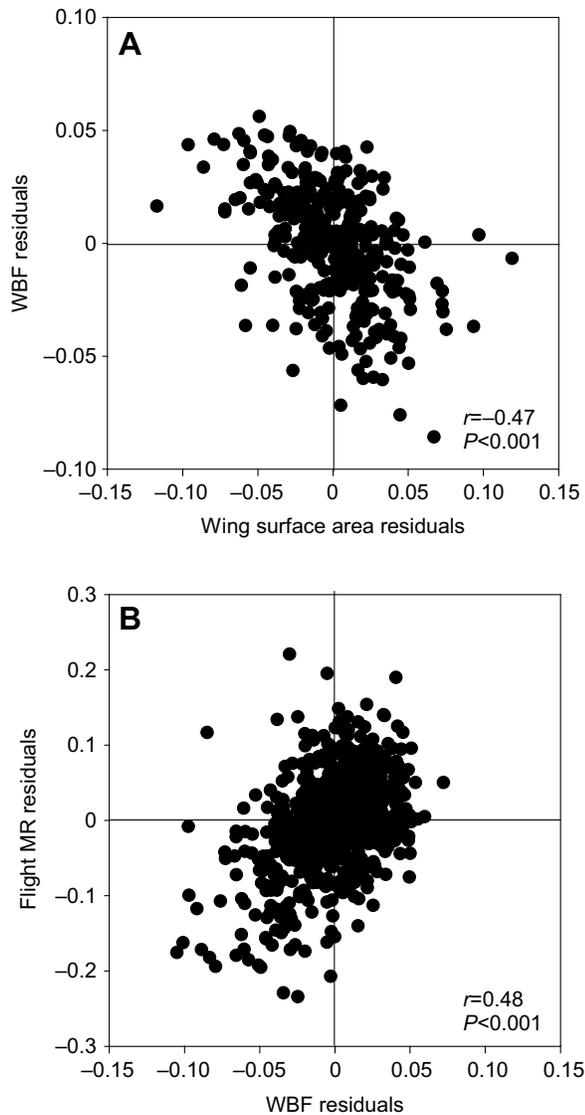
Fig. 3. Wing size, kinematics and flight MR are linked after accounting for body mass. Correlation between (A) WSA and WBF residuals (n=397) and (B) WBF and flight MR residuals (n=822) obtained from the mixed-effects models presented in Table 1.

For PGI, the younger queens had higher activity than the older gravid queens (Fig. 5D; $P<0.01$).

Family resemblance

For all traits measured, we found no significant relationships between the queen trait values and their offspring mean values. Nonetheless, interfamily differences for most traits were considerable. Differences among families were significant for all traits and intraclass correlation coefficients indicated that, for all traits studied, workers from the same colony tended to be more similar (Table 3).

DISCUSSION

Using female caste dimorphism, this study shows how body size impacts morphological and physiological traits linked with flight. The allometric relationships obtained for worker wing surface area, WBF, flight MR and RMR predict trait values of queens that are more than fourfold larger. Flight success of queens decreased over time, in

part because of a large increase in body mass and because of a decrease in traits linked with flight; namely, wingbeat frequency, metabolic rate and the activity of metabolic enzymes. Nonetheless, after accounting for temporal changes, traits were repeatable. We also found significant family resemblance for all traits measured, indicating that shared genes and/or environmental effects impact phenotypic variation. Together, these results show that the functional association between body morphology and flight physiology is robust, providing further insight into the mechanistic basis of MR scaling patterns during locomotion in animals.

Strictly based on wing surface area, *B. impatiens* queens are larger workers. In workers, wing surface area scales isometrically with body mass (Table 1), and queens fall mostly on (perhaps slightly below) the regression obtained from workers, and within the prediction intervals (based on WSA, Fig. 1A). Broad comparisons among species belonging to diverse families show that wing surface area scales isometrically with body mass (Byrne et al., 1988), although a substantial amount of variation remains. Comparison among closely related species of insects shows that wing surface area (Darveau et al., 2005a) and wing length depart from isometry (Sacchi and Hardersen, 2013), which is thought to be associated with differences in flight behaviour (Sacchi and Hardersen, 2013). Within species, diverse scaling trajectories of the wing–size relationship can evolve through artificial selection, but natural selection quickly returns to the initial allometric slope values (Bolstad et al., 2015). Our estimate of wing surface area scaling of *B. impatiens* based on a large number of workers shows isometric scaling, and reproductive queen caste is developing in the same morphospace.

From the perspective of flight kinematics, bumblebee queens also resemble workers, with WBF values that would be predicted from those of workers (Fig. 1C). Flight WBF is known to decrease with increasing body mass among insect species (Casey et al., 1985; Byrne et al., 1988; Darveau et al., 2005a) and within bumblebee species (Buchwald and Dudley, 2010; Skandalis and Darveau, 2012; van Roy et al., 2014). The relationship between flying animal body mass and WBF has been investigated in birds (Rayner, 1988; Pennycuick, 1990), mammals (Lindhe Norberg and Norberg, 2012) and insects (Byrne et al., 1988), and shows a consistent decrease with increasing body mass. According to the predictions from modelling of animal flight (Pennycuick, 1996, 2008; Deakin, 2010), geometrically similar animals should have a WBF scaling exponent of $-1/6$. Exponent values obtained for individual variation among workers that were found to be geometrically similar follows from the predicted values (-0.164, Table 1), adding to other reports on the same species using smaller sample sizes (Buchwald and Dudley, 2010; Skandalis and Darveau, 2012; Darveau et al., 2014). The functional association between wing size variation and kinematics is further supported by the relationship observed after accounting for body mass (Fig. 3A). Bumblebee queens falling on the regression line predicted from workers for wing size also fall on the predicted value for WBF, supporting the close association between flight form and function.

The link between flight WBF and flight MR has been investigated across insect species (Casey et al., 1985; Darveau et al., 2005a), which is predicted to follow from the flight muscle contraction frequency. Workers with a WBF allometric exponent of -0.164 would be predicted to have a mass-specific flight MR that follows the same exponent. The whole-animal scaling exponent of 0.829 (Table 1), when analysed and expressed on a mass-specific basis, yields a scaling value of -0.169 (data not shown), which essentially parallels the WBF exponent obtained, with queens mostly falling on the regression line and within the prediction interval (Fig. 1B).
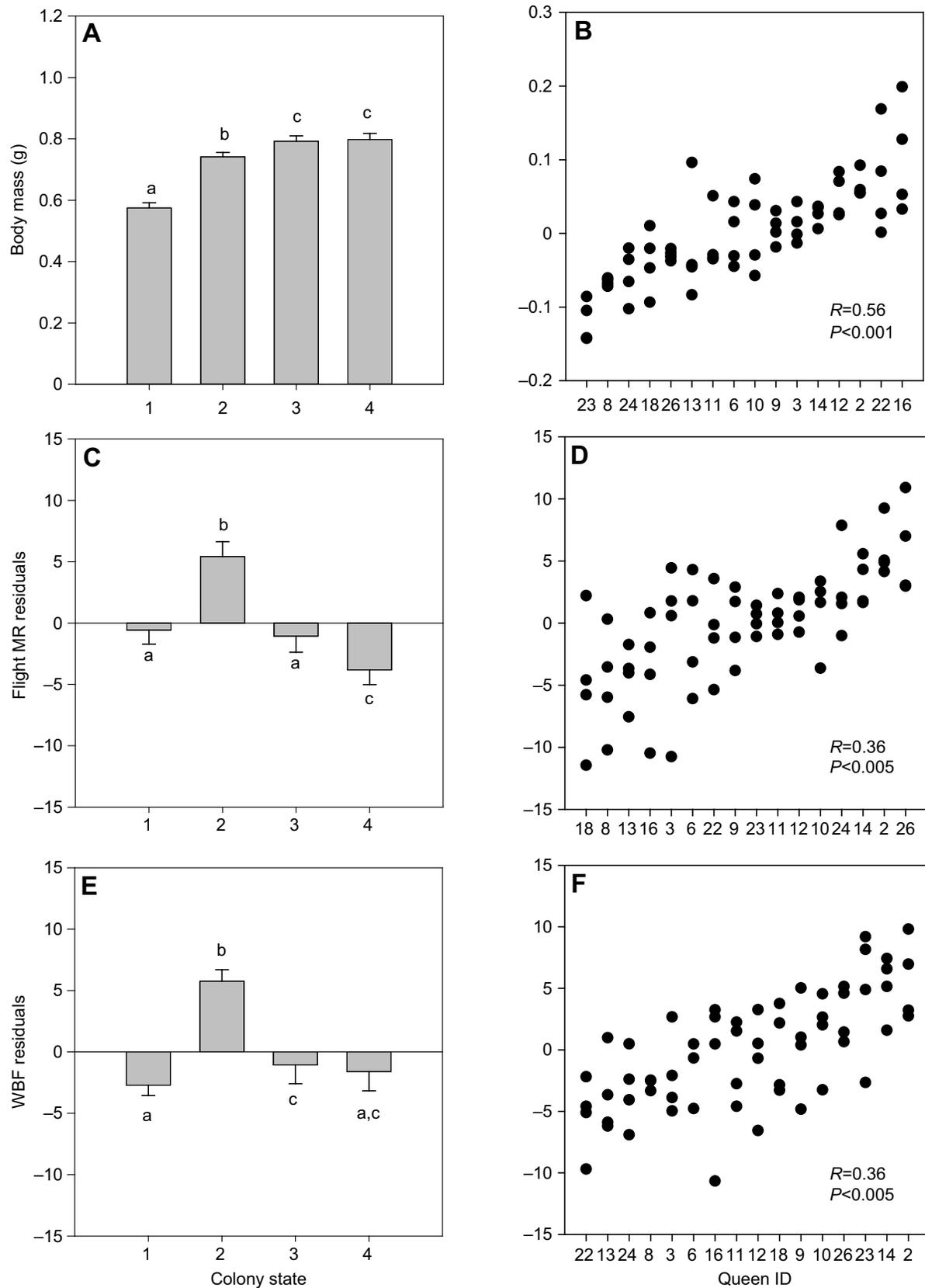
Fig. 4. Queen phenotype changes over time but traits remain repeatable. (A,C,E) Mean (±s.e.m.) values of body mass, flight MR residuals and WBF residuals from the body mass relationships of 16 *B. impatiens* queens at different states of their colony. The states presented are: (1) before the establishment of a colony, (2) 1 week after emergence of the first worker, (3) when the colony reached 30–40 workers and (4) 1 week after measurement 3. Groups that do not share letters are significantly different (*P*<0.05). (B,D,F) Caterpillar plots of queen body mass, flight MR residuals and WBF residuals further accounting for the state of the colony. Repeatability (*R*) was calculated from variance estimates obtained from mixed-effects models.
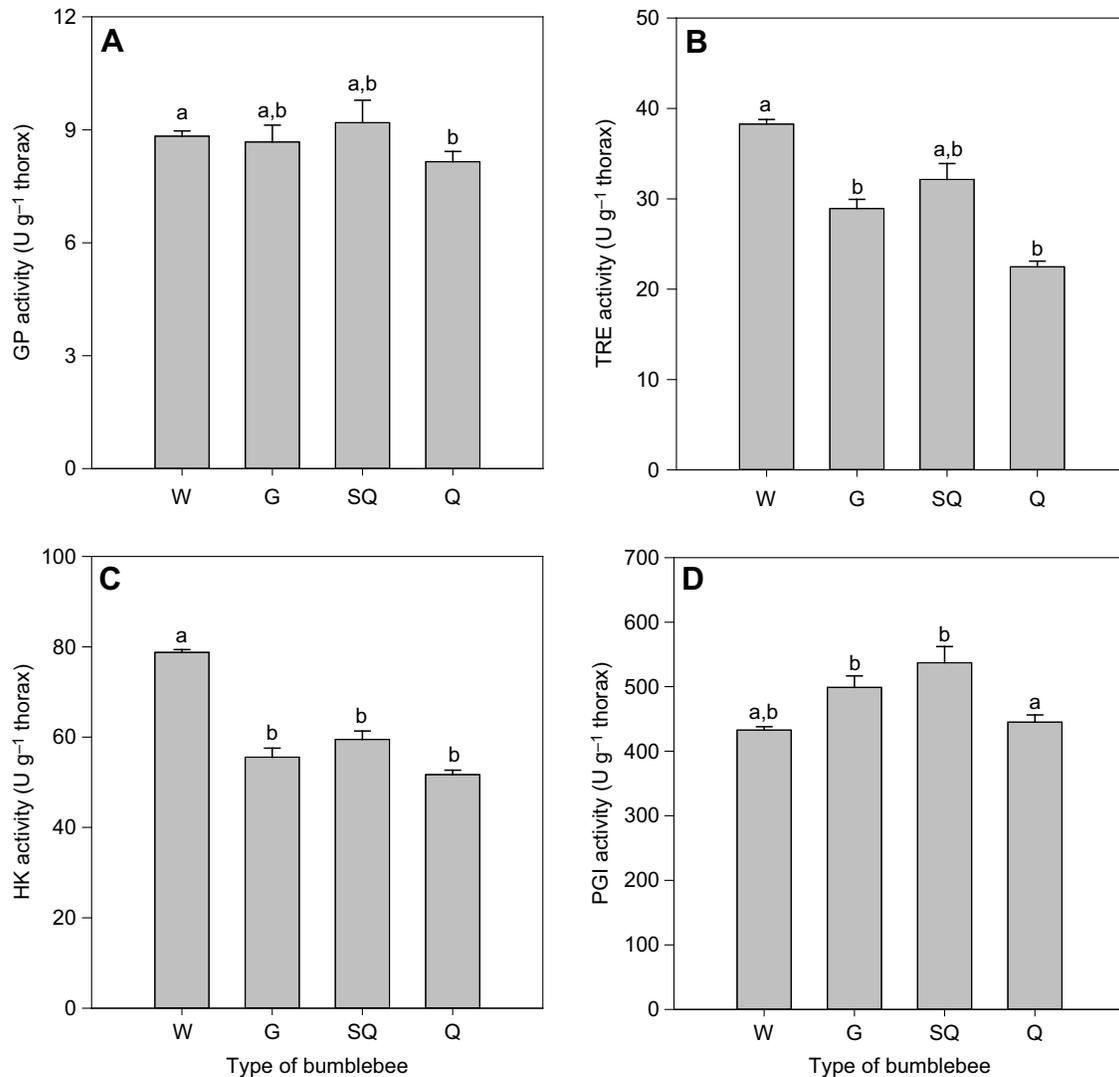
Fig. 5. Flight muscle enzyme activity differs between worker and queen bumblebees. Comparison of mean (±s.e.m.) (A) GP, (B) TRE, (C) HK and (D) PGI activity in workers (W; $n=306$), gynes (G; $n=30$), flying queens collected in the spring (SQ; $n=14$) and gravid queens (Q; $n=52$). Differences between types of bees (W, G, SQ and Q) were determined using mixed-effects models including the fixed effects population of origin, body mass and type. Groups that do not share letters are statistically different ($P<0.05$).

Furthermore, association between traits that are independent of body mass is supported (Fig. 3B), strongly linking variation in frequency to variation in MR (Skandalis and Darveau, 2012; Darveau et al., 2014). It therefore appears clear to us that flight MR scaling can be predicted from the wing form to flight kinematics association, predicting functional phenotypes of insect castes (Darveau et al., 2014). We complemented the flight energetic measurements with RMR values which also scale allometrically with worker body mass with a similar exponent value (Table 1, Fig. 1D); once again, queen values can be predicted from the relationship obtained from workers. The drivers of RMR are much more elusive (Reinhold, 1999; Chown et al., 2007; Waters and Harrison, 2012), but its association with activity MR values has been hypothesized but remains to be properly studied with paired measurements.

Flight muscle metabolic properties of queens, as described by the activity of metabolic enzymes, are distinct from those of workers. Although the activity of most enzymes was not associated with worker body mass (Table 2, Fig. 2), larger queens have lower activities of enzymes involved in carbohydrate metabolism (Fig. 5).

This is indeed the prediction from differences across bee species (Darveau et al., 2005b), but also from intraspecific studies on *B. impatiens* comparing male with female worker castes (Darveau et al., 2014). These intraspecific patterns are in line with broad interspecific studies in vertebrates where aerobic metabolic enzyme activity tends to scale similarly to MR (Somero and Childress, 1980; Emmett and Hochachka, 1981). The relatively narrow range in body mass combined with variable activity within workers means there was no clear association with body mass, although this was significant for the enzyme HK, but the much larger size of queens extends the size range such that flight muscle enzyme activity is lower compared with that of workers (Figs 2 and 5).

Flight muscle and whole-animal metabolic phenotype change over time. From the time queens establish their nest to the time their nest contains over 40 workers, queens increase substantially in body mass, probably coinciding with the transition from establishing and provisioning their nests with resources to strictly egg-laying and nest maintenance tasks, periods during which substantial differences in physiological state should occur (Amsalem et al., 2015).

Table 3. Family resemblance estimated using intraclass correlation coefficient analysis

Trait	Family mean value range	N	n	Intraclass correlation coefficient
Morphological				
Body mass (g)	0.143–0.212	54	822	**0.131**
WSA (mm^2)	4.97–7.06	43	397	**0.249**
Physiological				
Flight MR (ml CO$_2$ h^{-1})	10.82–15.85	54	822	**0.073**
RMR (ml CO$_2$ h^{-1})	0.23–0.39	25	195	**0.254**
WBF (Hz)	182–206	54	822	**0.128**
Biochemical				
GP (U g^{-1})	4.77–12.08	46	256	**0.175**
TRE (U g^{-1})	24.86–49.12	46	256	**0.255**
HK (U g^{-1})	58.00–84.96	46	256	**0.187**
PGI (U g^{-1})	293.82–544.52	46	256	**0.242**

GP, glycogen phosphorylase; TRE, trehalase; HK, hexokinase; PGI, phosphoglucoisomerase. Note: coefficients were obtained from the variance components reported from the mixed-effects models presented in Tables 1 and 2. The range of family means is presented for each trait. The number of families (N) and the total number of individuals (n) are also shown. Values significant at α=0.05 are in bold.

The increase in body mass was detected at our first measurement conducted 1 week after the emergence of the first worker, which coincides with a substantial increase in flight WBF and MR independent of body mass. Our interpretation of these results is that this period corresponding with a large increase in egg mass, and possibly energy reserves, could require a compensatory increase in flight energetics parameters. Interestingly, honeybee workers show no detectable increase in flight WBF (or stroke amplitude) during pollen or nectar load carrying, and only a small increase in flight MR could be detected (Feuerbacher et al., 2003), which was also noted to a greater extent by Wolf et al. (1989). Nevertheless, the increase in flight properties is transient and not maintained over the following periods where the colony size increased to 40 workers and more. Measurements plotted in Fig. 1 were conducted on queens at the 40 worker stage; therefore, prior to establishing their colony, younger queens would have lower body mass, higher flight MR and similar WBF, but still within the prediction limits obtained from workers (data not shown). Queens that have well-established colonies no longer have to maintain flight; in fact, we observed that successful flights become difficult to achieve and substantial wing wear prevented us from measuring wing surface area of some older queens. There is a deterioration of flight capacity and properties in older queens, which tends to be observed at the level of flight muscle metabolic enzyme activity.

Flight performance of queens declines over time but, after accounting for this, body mass, flight MR and WBF are nevertheless repeatable. This is in agreement with observations made on workers of the same species (Darveau et al., 2014), supporting that features such as individual variation in morphology and physiology impact flight energetics in a consistent manner. The significant repeatability estimates potentially suggest some level of heritability of morphological and physiological traits associated with flight. We found that families differed and siblings resembled each other for all phenotypes measured. Sibling resemblance can be explained by shared genetic variation, including additive genetic variation, and/or environmental effects. Additionally, there was a clear distinction in some metabolic phenotypes between commercial and wild populations of *B. impatiens* (Tables 1 and 2), suggesting a genetic basis for metabolic phenotype variation or that a common environment before establishment of the colony impacted muscle metabolic properties. The potential that some of the observed phenotypic variation is due to additive genetic effects is supported by several studies showing significant heritability of flight-associated phenotypes in many insect species, including wing morphology in fruit flies (Curtsinger and Laurie Ahlberg, 1981; Moraes and Sene, 2004), crickets (Bégin and Roff, 2002) and moths (Keena et al., 2007), various flight performance parameters such as flight distance and duration in moths (Parker and Gatehouse, 1985; Gu and Danthanarayana, 1992; Han and Gatehouse, 1993; Schumacher et al., 1997), beetles (Tanaka, 2009) and fruit flies (Gu and Barker, 1995), and flight metabolic rate in butterflies (Mattila and Hanski, 2014). Furthermore, the significant heritability of the activity of enzymes has also been shown in *Drosophila melanogaster* (Laurie-Ahlberg et al., 1982; Pecsenye et al., 2004). Our results on family resemblance combined with evidence from the literature suggest some degree of heritability for flight-related morphological and physiological parameters measured.

In summary, the intraspecific scaling of flight MR in an insect species could be explained by the functional links between flight form and function, as predicted from interspecific studies. Large differences in body mass of the queen caste of social bumblebees further allows testing of the predicted impact of size on flight metabolic properties, including the activity of some metabolic enzymes in flight muscle. Furthermore, family resemblance suggests the potential heritabilty of morphological and physiological traits linked with flight; natural selection could lead to the correlated evolution of traits, as suggested from macroevolutionary studies (Darveau et al., 2005a,b; Rodriguez et al., 2015). Flying insects serve as a great example of how principles of scaling of animal locomotion, within and among species, impact MR scaling and tissue properties.

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