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

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Summary statement

Comparing workers and queens bumblebee reveals how body size impact flight energetics and muscle metabolism.

Abstract

Animal size affects energetics of locomotion. Using female caste dimorphism in bumblebees, we assessed how body mass impacted morphological and physiological traits linked with flight. The allometric relationships obtained for workers wing surface area, wingbeat frequency, flight and resting metabolic rates could predict the trait values of queens that are more than four-fold larger. Flight success of queens decreased over time in part due to a large increase in body mass, and decrease in traits linked with flight, namely wingbeat frequency, 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, we here 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.

Introduction

The effect of animal size on biological form and function is profound and diverse. The impact of body size on animal locomotion has been and 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 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 have 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, draw mechanistic explanation from the observed relationships, and test 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 constraint of the tracheal system on oxygen delivery (Keiser et al. 2007), the physiological and biochemical determinants of flight metabolic rate (Casey et al. 1985, Darveau et al. 2005a, Darveau et al., 2005b; Rodriguez et al. 2015), or the impact of animal size on the biomechanics of flight (Casey et al., 1992; Dillon and Dudley, 2004). Interindividual variation has been useful to study changes in respiratory properties during instar transitions in hemimetabolous insects (Greenlee et al., 2009; Snelling et al., 2011, 2012). Holometabolous insects can exhibit a large enough body size range to investigate 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). Few studies to date have, however, 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 to test predicted impact on their function, and ultimately bridge the gap between inter and intraspecific scaling studies.

Insects body size affects flight kinematics, which in turn drives variation in 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 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, which is likely 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 association was also found among individuals within a species (Skandalis and Darveau, 2012; Darveau et al, 2014).

The use of large variation in body mass found within species can be a powerful tool to test hypothesized association among functional traits that are often derived from interspecific studies. The group of hymenopterans can be especially useful given that caste polymorphism is found in some social species. Bumblebees such as *B. impatiens* has a wide distribution in body size among workers (Couvillon et al., 2010), but in addition queens can be much larger than workers and drones (del Castillo and Fairbairn, 2011). Based on previous work investigating determinants of flight energetics between species (Darveau et al., 2005a; Darveau et al., 2005b) and among *B. impatiens* individuals (Skandalis and Darveau, 2012; Darveau et al., 2014), we hypothesize that large bumblebee queens will have lower wingbeat frequency and mass-specific flight metabolic rate. Secondly, flight muscles metabolic enzyme activities should be lower in queens given its larger size and predicted lower mass-specific metabolic rate. Finally, data generated on queens and offspring workers allow to test family resemblance, thereby providing insights 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 (Quebec, Canada) region. Commercial colonies were kept in the suppliers housing boxes in a room maintained at around 25˚C with a 12L:12D photoperiod. Colonies were provided with unlyophilized pollen and sucrose solution (50% v/v) *ad libitum* and acclimated to these laboratory conditions for one 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 12L:12D 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 (FlightMR) and wingbeat frequency (WBF) measurements were performed on each queen and a subset of 15 of its 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 during the progression of the colony development. A first measurement 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, Las Vegas, Nevada; SSI thereafter) linked to a temperature controller (Pelt-5, SSI) and maintained at 22±2^{o}C. The second measurements were conducted one week after the first workers had hatched in the laboratory. The third series of measures was performed after 40 workers were present in the colony, which coincide with the size of the commercial colonies when measurements were performed. A final set of measurements was obtained one week later. All individuals were then dissected and stored at -80˚C for morphological and cellular measurements.

Rates of CO_2 production and wingbeat frequency were measured as described in other studies (Skandalis et al. 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 5 PM and 9 AM. 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 in 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 analyzer (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 baseline CO_2 measurement. Each bee was measured for 1 hr and CO_2 baseline was monitored before and after each individual. 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 anesthetized 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. Since wing venation is conserved within Hymenoptera species (Francoy et al., 2009), we measured a wing section which 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 analyses

Statistical analyses were performed using the software SYSTAT 13.0 (Chicago, 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 relationship 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 colonies. 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, their standard error, F statistics and corresponding P-values are reported. The β 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 are 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 chi2 distribution with a 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 differences in enzyme activities 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.

In order to evaluate the phenotypic variation of body mass and flight performance traits (FlightMR, WBF) in queens, we performed mixed effects model 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, one week after the emergence of the first worker, when the colony reached 40 workers and one week after the latter. Repeatability was calculated from the variance parameters obtained from the mixed effects models and significance 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 if 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 Table 1 and 2. We also used the intra-class correlation coefficient to determine if 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 $\chi^2_{0.5}$ 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 wingbeat frequency 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 workers body mass on morphological and energetic parameters is summarized in Table 1. Wing surface area scaled isometrically with an exponent value of 0.668; the wing section area selected for estimation of surface area for damaged wing individual scaled with slightly lower exponent (b = 0.637). Flight and resting metabolic rates scaled allometrically with workers body mass with exponent values of 0.829 and 0.867, respectively. Wingbeat frequency decreased with increasing body mass with scaling exponent of -0.164. Variation in wing surface area and wingbeat frequency, after accounting for body mass, was negatively correlated, and flight metabolic rate and wingbeat frequency was positively correlated (Fig. 3).

Measurements performed on queens showed that wild population had higher FlightMR than 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 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 wing section area (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 queens' phenotypes change over time. Queen mass increased significantly from the time of capture before the establishment of the colony to one week after the emergence of the first worker, and further increase by the time the colony is well established with over 40 workers (Fig. 4A). After accounting for body mass, flight metabolic rate and wingbeat frequency increased from the time of capture to the time the colony is just established, and further declined as the colony development progressed (Fig. 4C and E). Although queens' body mass, flight metabolic rate and wingbeat frequency changed over time, after accounting for temporal changes all traits remained repeatable (Fig. 4 B, D, F).

We compared workers with gynes in the laboratory, young queens collected in the spring, and gravid queens with established colonies (Figure 5). Using mixed models with population of origin, body mass and bee type as fixed effects, analyses revealed that workers had higher GP activities than gravid queens (Fig. 5A; $P = 0.037$). The activity of TRE and HK was also the lowest in gravid queens and different than workers (Fig. 5B,C; $P < 0.01$). For PGI, the younger queens had higher activities than the older gravid queens Fig. 5D; ($P < 0.01$).

Family ressemblance

For all traits measured, we found no significant relationships between the queens trait values and their offsprings' mean values. Nonetheless, interfamily differences for most traits were considerable. Differences among families was significant for all trais and intraclass correlation coefficient indicate that for all traits studied workers from the same colony tend 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 workers wing surface area, wingbeat frequency, flight and resting metabolic rates predict the trait values of queens that are more than 4-fold larger. Flight success of queens decreases over time and is in part due to a large increase in body mass, and decrease in traits linked with flight, namely wingbeat frequency, metabolic rate, and the activity of metabolic enzymes. Still, 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, we here 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.

Strictly based on wing surface area, *B. impatiens* queens are larger workers. In workers, the wing surface area scale isometrically with body mass (Table 1), and queens fall mostly on the regression obtained from workers, maybe slightly below, and within the prediction intervals (based on wing section area, 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 substantial amount of variation remains. Comparison among closely related species of insects show that wing surface area (Darveau et al., 2005a) and wing length depart from isometry (Sacchi and Hardersen, 2013), which is thought 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 queens caste is developing in the same morphospace.

From the perspective of flight kinematic, bumblebee queens also resemble workers, with wingbeat frequency values that would be predicted from workers (Fig. 1C). Flight wingbeat frequency 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 (Bushwald and Dudley, 2010; Darveau and Skandalis, 2012; van Roy et al., 2014). The relationship between flying animals body mass and wingbeat frequency has been investigated in birds (Rayner, 1988; Pennycuick, 1990), mammals (Lindhe Norberg and Norberg, 2012), and insects (Byrne et al. 1988), showing consistent decrease with increasing body mass.

According to the predictions from modelling of animal flight (Pennycuick, 1996; Pennycuick, 2008; Deakin, 2010), geometrically similar animals should have wingbeat frequency 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 kinematic is further supported from 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 wingbeat frequency, supporting the close association between flight form and function.

The link between flight wingbeat frequency and flight metabolic rate 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 wingbeat frequency allometric exponent of -0.164 would be predicted to have mass-specific flight metabolic rate that follows the same exponent. The whole-animal scaling exponent of 0.829 (Table 1), when analyzed and expressed on a mass-specific basis yields a scaling value of -0.169 (data not shown), which essentially parallels the wingbeat frequency exponent obtained, with queens mostly falling on the regression line and within the prediction interval (Fig. 1B). Furthermore, association between traits that is independent of body mass is supported (Fig. 3B), strongly linking variation in frequency to variation in metabolic rate (Skandalis and Darveau, 2012; Darveau et al., 2014). It therefore appears clear to us that flight metabolic rate scaling is 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 resting metabolic rate values which also scale allometrically with workers body mass with similar exponent value (Table 1, Fig. 1D); once again, queen values can be predicted from the relationship obtained from workers. The drivers of resting metabolic rate are much more elusive (Reinhold, 1999; Chown et al., 2007; Waters and Harrison, 2012), but its association with activity metabolic rate 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 workers. Although the activity of most enzymes was not associated with workers 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 instrapecific 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 enzymes tend to scale similarly to metabolic rate (Somero and Childress, 1980; Emmett and Hochachka, 1981). The relatively narrow range in body mass combine with variable activities within workers does not clearly exhibit the association with body mass, although significant for the enzyme hexokinase, but the much larger size of queens extend the size range such that flight muscles enzyme activity is lower compared with workers (Fig. 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, likely coinciding with the transition between establishing and provision their nests with resources, to a strictly egg-laying and nest maintenance tasks, periods during which substantial differences in physiological state should occur (Amsalem et al., 2015). The increase in body mass is detected at our first measurement conducted one week after the emergence of the first worker, which coincides with a substantial increase in flight wingbeat frequency and metabolic rate 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 compensatory increase in flight energetics parameters. Interestingly, honeybee workers show no detectable increase in flight wingbeat frequency (or stroke amplitude) during pollen or nectar load carrying, and only small increase in flight metabolic rate 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 increase to 40 workers and more. Measurements plotted on Figure 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 metabolic rate and similar wingbeat frequency, 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 tend to be observed at the level flight muscle metabolic enzyme activity.

Flight performance of queens decline over time, but after accounting for it, body mass, flight metabolic rate and wingbeat frequency 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 family differed and sibblings resemble 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 is a clear distinction in some metabolic phenotypes between commercial and wild populations of *B. impatiens* (Table 1 and Table 2), suggesting a genetic basis of metabolic phenotypes variation or that 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), a cricket (Bégin and Roff, 2002) and a moth (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) a beetle (Tanaka, 2009) and fruit flies (Gu and Barker, 1995), and flight metabolic rate in a butterfly: 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 makes us suggest some degree of heritability for flight related morphological and physiological parameters measured.

In summary, the intraspecific scaling of flight metabolic rate 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 to test the predicted impact of size on flight metabolic properties, including the activity of some metabolic enzyme in the flight muscle. Furthermore, family resemblance suggest 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; Darveau et al., 2005b; Rodriguez et al., 2015). Flying insects serve as great example demonstrating how principles of scaling of animal locomotion, within and among species, impact metabolic rate scaling and tissue properties.

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Competing interests

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

Body mass (g)	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	1.150×10^{-3}	48.94	<0.001
	Residuals	7.606×10^{-3}		
WSA (mm^2)	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*P*
	Intercept	1.987 (0.013)	$23432.02_{1,\ 41}$	<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	$X^2_{0.5}$	
	Colony	2.36×10^{-4}	48.14	<0.001
	Residuals	7.12×10^{-4}		
WSectA (mm^2)	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	1.63×10^{-4}	16.56	
	Residuals	1.07×10^{-3}		
FlightMR (ml CO_2 hr^{-1})	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	3.35×10^{-4}	19.54	<0.001
	Residuals	4.27×10^{-3}		
RMR (ml CO_2 hr^{-1})	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	2.24×10^{-3}	26.73	<0.001
	Residuals	6.59×10^{-3}		
WBF (Hz)	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*P*

Intercept	2.172 (0.008)	$68212.59_{1,52}$	<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	$X^2_{0.5}$	
Colony	1.00×10^{-4}	45.60	<0.001
Residuals	6.79×10^{-4}		

Note: All variable 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.

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

GP (U g^{-1})	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	2.22×10^{-3}	11.69	<0.001
	Residuals	1.04×10^{-2}		
TRE (U g^{-1})	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	2.05×10^{-3}	23.24	<0.001
	Residuals	5.99×10^{-3}		
HK (U g^{-1})	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	4.41×10^{-4}	13.86	<0.001
	Residuals	1.92×10^{-3}		
PGI (U g^{-1})	Fixed	β(SE)	$F_{(\mathrm{NUMdf,\ DENdf})}$	*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	$X^2_{0.5}$	
	Colony	1.76×10^{-3}	22.72	<0.001

Residuals	5.53×10^{-3}	—

Note: All variable 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 the difference in slope for commercial colonies.

Table 3 Family resemblance estimated using intraclass correlation coefficient analysis.

Trait	Family mean value range	N	*n*	Intraclass correlation coefficient
Morphological trait				
Body Mass (g)	0.143 - 0.212	54	822	**0.131**
WSA (mm^2)	4.97 - 7.06	43	397	**0.249**
Physiological trait				
FlightMR (ml CO_2 hr^{-1})	10.82 - 15.85	54	822	**0.073**
RMR (ml CO_2 hr^{-1})	0.23 – 0.39	25	195	**0.254**
WBF (Hz)	182- 206	54	822	**0.128**
Biochemical trait				
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**

Note: Coefficient were obtained from the variance components reported from the mixed-effects models presented in Table 1 and 2. Range of family means is presented for each trait. The number of families (N), the total number of individuals (*n*) are also shown. Values significant at $\alpha = 0.05$ are marked in bold.

Figure 1. The effects of body mass on traits of workers and queens bumblebee. Relationship between body mass (g) and (A) wing section area (mm^2) (workers: n = 397; queens: n = 42), (B) flight metabolic rate (ml CO_2 hr^{-1}) (workers: n = 822; queens: n = 42), (C) wing beat frequency (Hz) (workers: n = 822; queens: n = 42) and (D) resting metabolic rate (ml CO_2 hr^{-1}) (workers: n = 195; queens: n = 24) in commercial (closed circles) and wild (open circles) *B. impatiens* workers and larger size queens. Regression lines represented are for linear regressions performed on workers only and extended to axes to locate queens in relation to the predictions from workers relationships. 95% confidence (solid interval lines) and prediction (dashed interval lines) intervals are presented. Additional variables tested using mixed effect models are presented in Table 1.

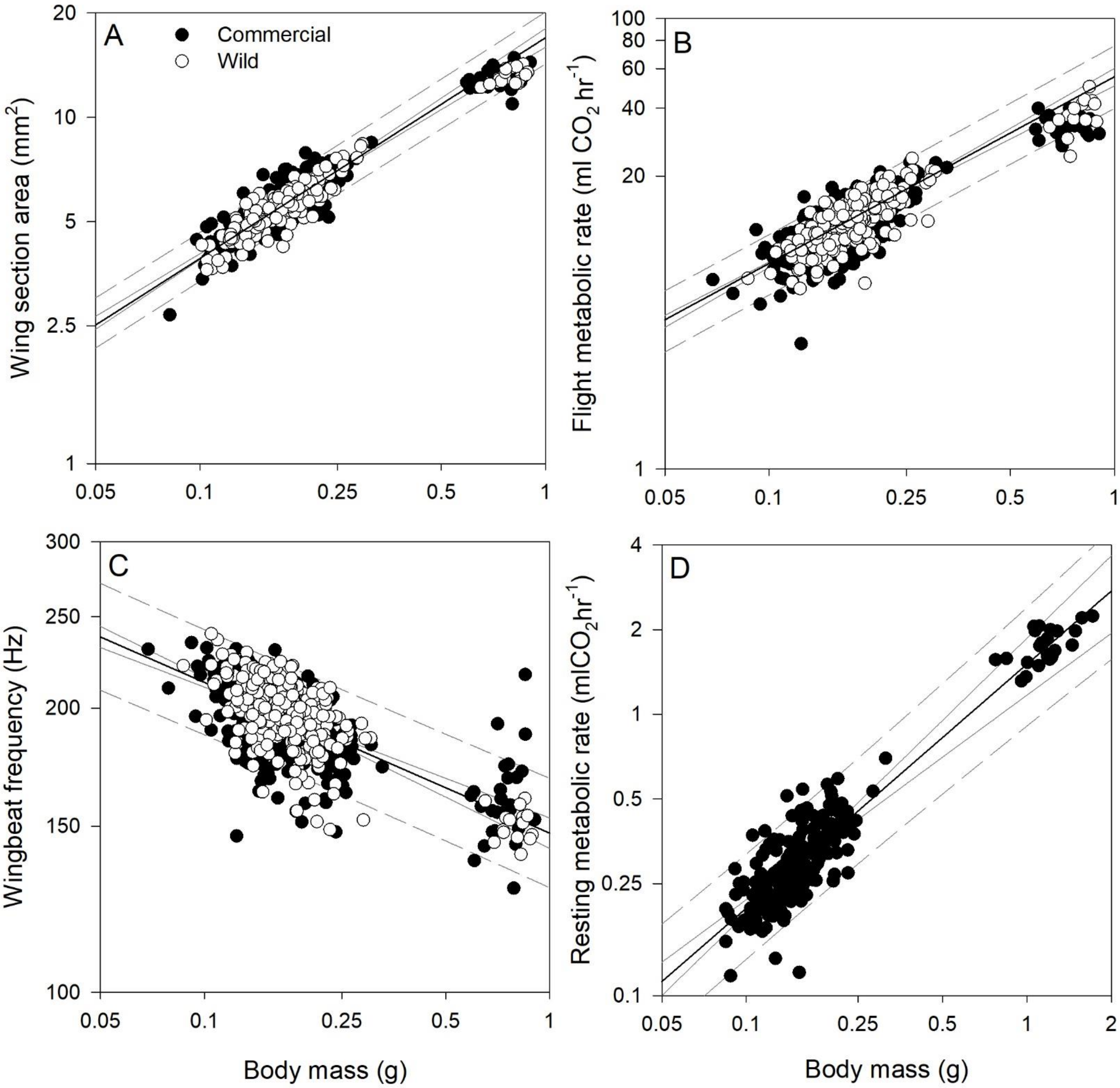
Figure 2. Flight muscle enzyme activity in workers and queens bumblebee. Relationship between body mass (g) and the activity (U g^{-1} thorax) of (A) glycogen phosphorylase (GP), (B) trehalase (TRE), (C) hexokinase (HK) and (D) phosphoglucoisomerase (PGI) in commercial (closed circles) and wild (open circles) *B. impatiens* workers (n=306) and queens (n=52). Sources of variation in enzyme activities of workers obtained from mixed effects models are reported in Table 2. For HK, regression is for commercial workers only using parameters from Table 2, and extented to axes to locate queens in relation to the predictions from the workers relationship.

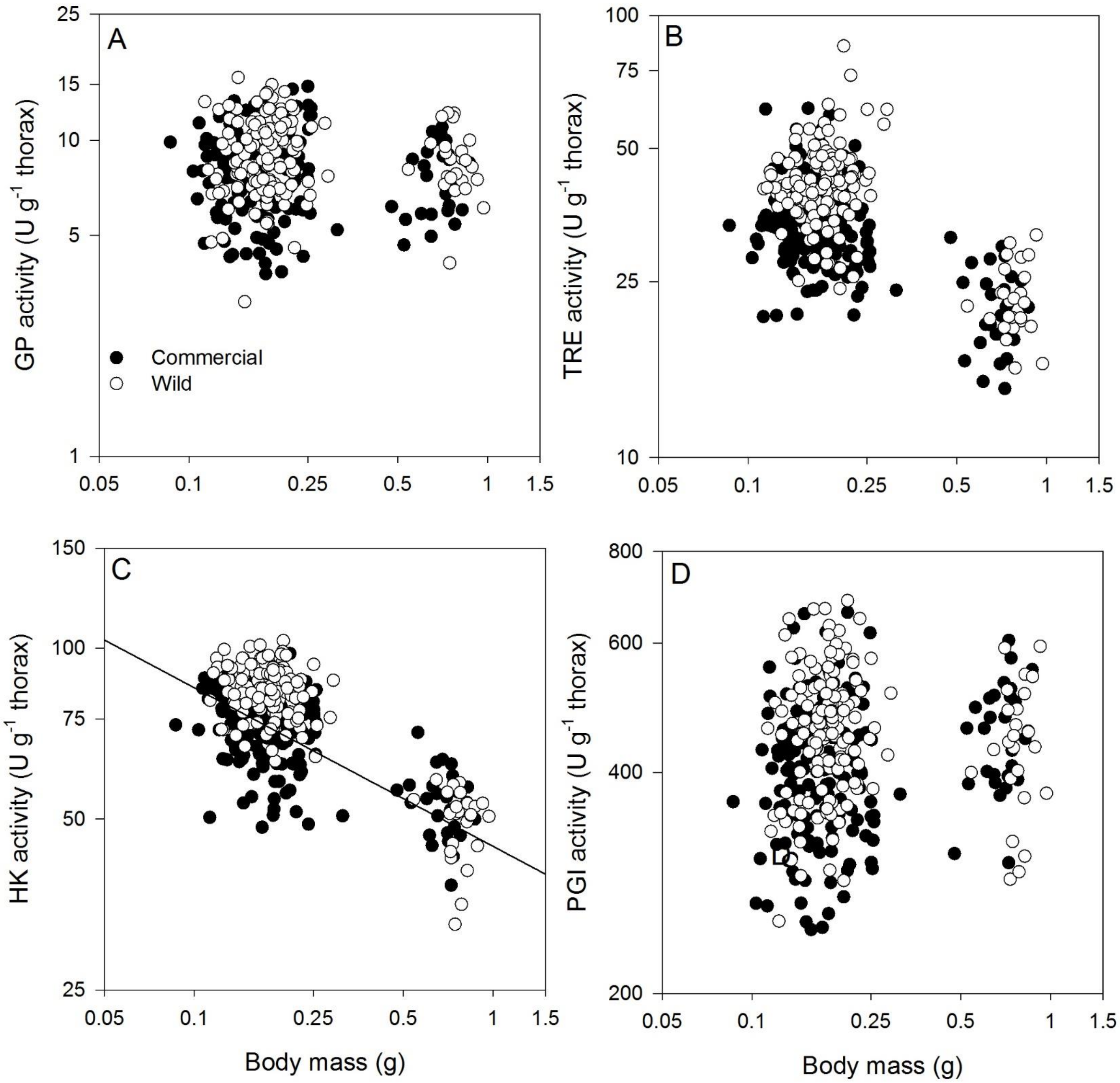
Figure 3. Wing size, kinematics and flight metabolic rate are linked after accounting for body mass. Correlation between (A) wing section area and wingbeat frequency residuals (n=397) and (B) wingbeat frequency and flight metabolic rate residuals (n=822) obtained from the mixed effects models presented in Table 1.

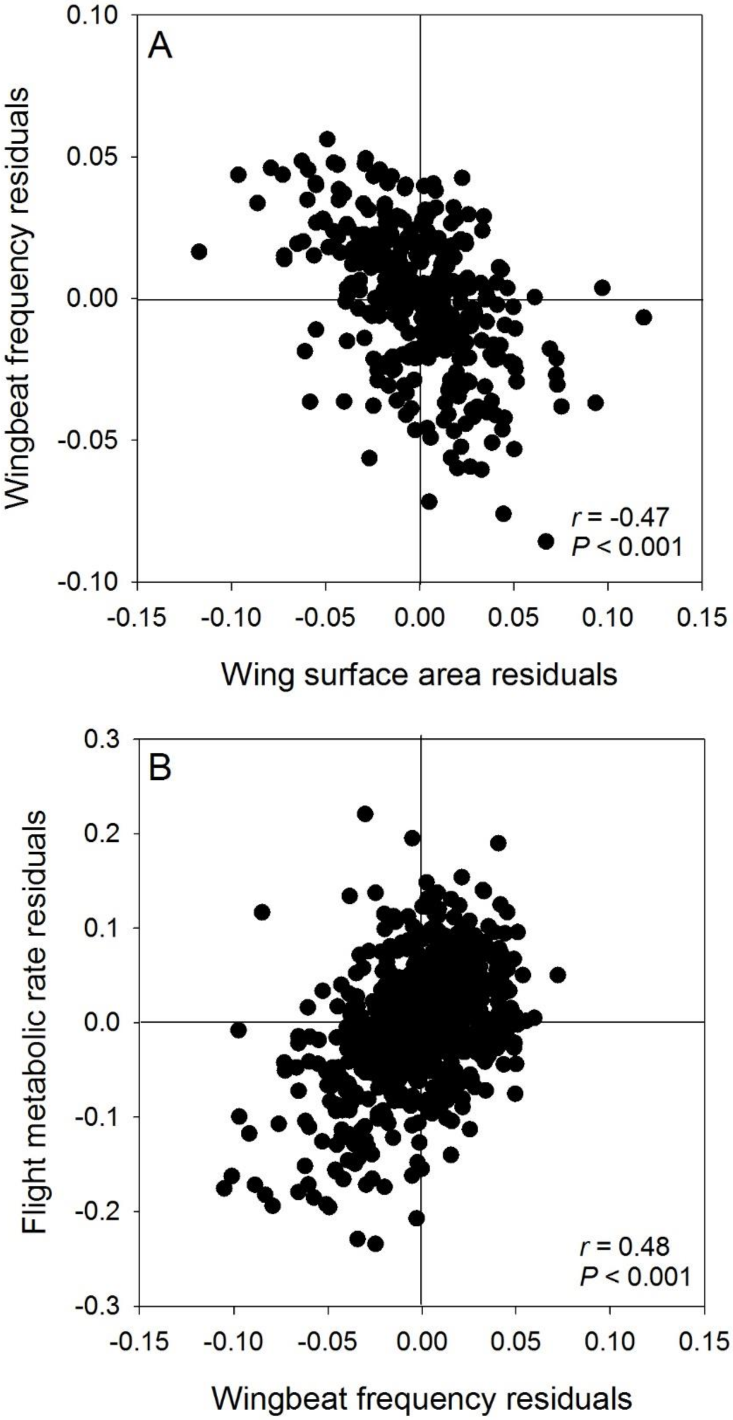
Figure 4. Queens change over time but traits remain repeatable. (A, C, E) Mean values (±s.e.m.) of body mass (g), flight metabolic rate residuals (FlightMR) (ml CO_2 hr^{-1}), and wing beat frequency (WBF) (Hz) residuals from the body mass relationships in 16 *B. impatiens* queens at different states of their colony. The states presented are: 1) before the establishment of colony, 2) one week after emergence of first worker, 3) when the colony reached 30-40 workers, and 4) one week after measurement 3. Groups that do not share letters are significantly different ($p<0.05$). (B, D, F) Caterpillar plots of queen's body mass, flight metabolic rate residuals, and wingbeat frequency residuals further accounting for state of the colony. Repeatability (*R*) was calculated from variance estimates obtained from mixed effects models.

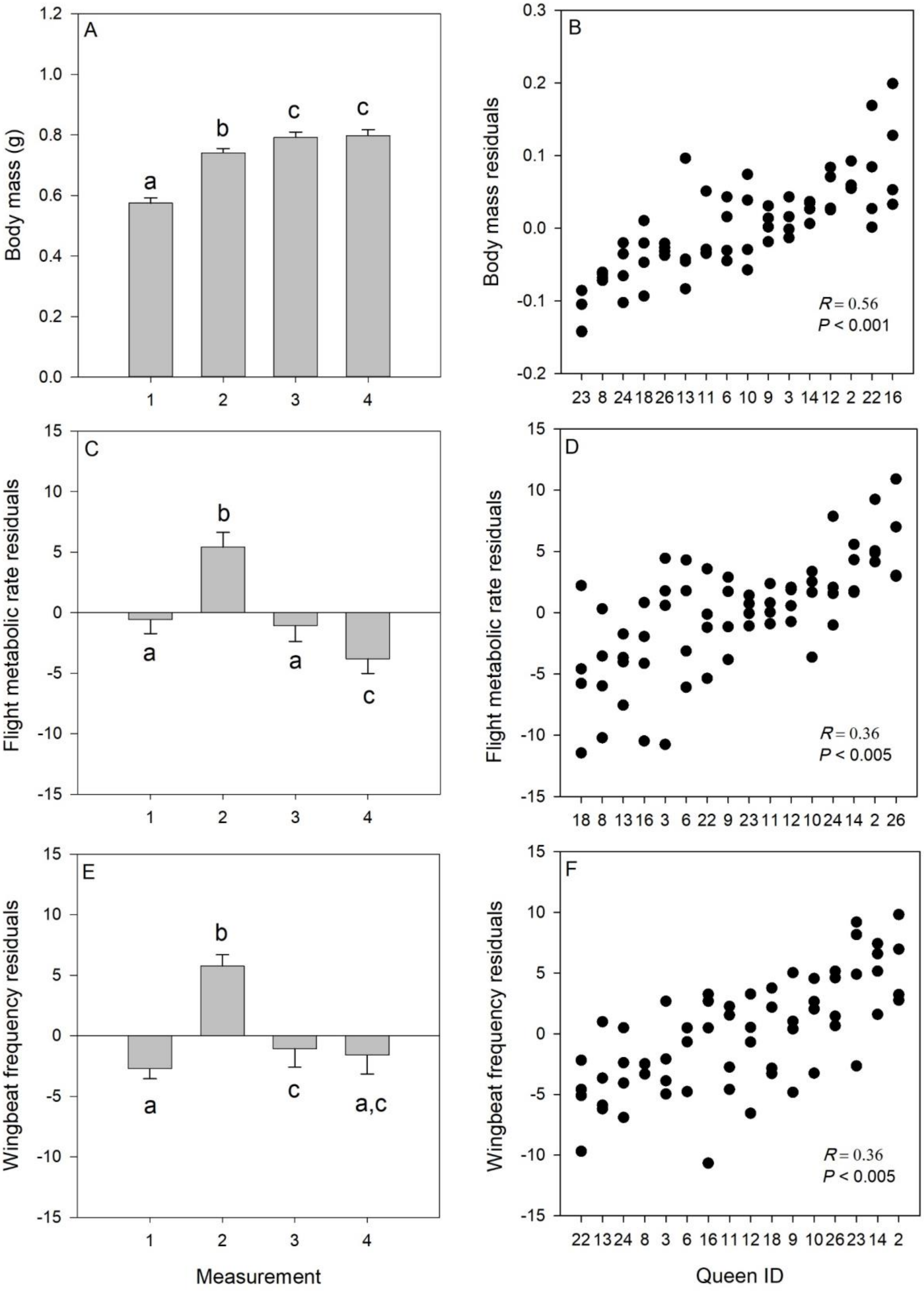
Figure 5. Flight muscle enzyme activity differ between workers and queens bumblebee. Comparison of mean (±s.e.m.) (A) glycogen phosphorylase (GP), (B) trehalase (TRE), (C)

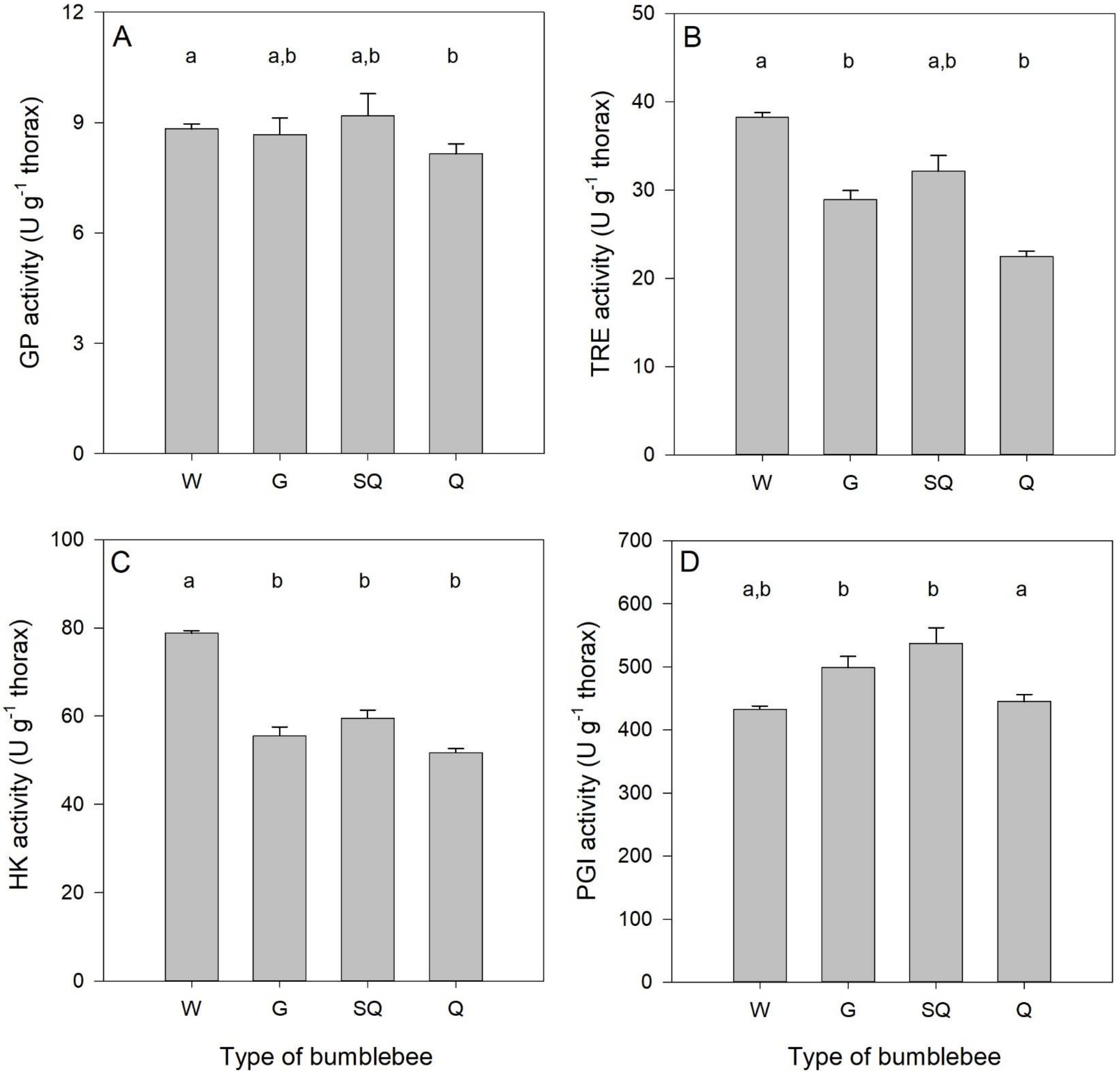
hexokinase (HK) and (D) phosphoglucoisomerase (PGI) activities (U g^{-1} thorax) in workers (W) (n=306), gynes (G) (n=30), flying queens collected in the spring (SQ) (n=14) and gravid queens (Q) (n=52). Differences between types of bees (W, G, SQ and Q) were determined using mixed-effects models including the fixed effects population of origin, body mass, and type. Groups that do not share letters were statistically different ($p<0.05$).

A
● Commercial
○ Wild
Wing section area (mm^2)
20
10
5
2.5
1
0.05
0.1
0.25
0.5
1
B
Flight metabolic rate (ml CO_2 hr^{-1})
100
80
60
40
20
1
0.05
0.1
0.25
0.5
1
C
Wingbeat frequency (Hz)
300
250
200
150
100
0.05
0.1
0.25
0.5
1
Body mass (g)
D
Resting metabolic rate (ml$CO_2$$hr^{-1}$)
4
2
1
0.5
0.25
0.1
0.05
0.1
0.25
0.5
1
2
Body mass (g)


A
25
15
10
5
1
GP activity (U g^{-1} thorax)
● Commercial
○ Wild
0.05
0.1
0.25
0.5
1
1.5
B
100
75
50
25
10
TRE activity (U g^{-1} thorax)
0.05
0.1
0.25
0.5
1
1.5
C
150
100
75
50
25
HK activity (U g^{-1} thorax)
0.05
0.1
0.25
0.5
1
1.5
Body mass (g)
D
800
600
400
200
PGI activity (U g^{-1} thorax)
0.05
0.1
0.25
0.5
1
1.5
Body mass (g)


A
Wingbeat frequency residuals
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
Wing surface area residuals
r = -0.47
P < 0.001
B
Flight metabolic rate residuals
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
Wingbeat frequency residuals
r = 0.48
P < 0.001


A
Body mass (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
c
c
1
2
3
4
B
Body mass residuals
0.3
0.2
0.1
0.0
-0.1
-0.2
$R = 0.56$
$P < 0.001$
23 8 24 18 26 13 11 6 10 9 3 14 12 2 22 16
C
Flight metabolic rate residuals
15
10
5
0
-5
-10
-15
b
a
a
c
1
2
3
4
D
Flight metabolic rate residuals
15
10
5
0
-5
-10
-15
$R = 0.36$
$P < 0.005$
18 8 13 16 3 6 22 9 23 11 12 10 24 14 2 26
E
Wingbeat frequency residuals
15
10
5
0
-5
-10
-15
b
a
c
a,c
1
2
3
4
Measurement
F
Wingbeat frequency residuals
15
10
5
0
-5
-10
-15
$R = 0.36$
$P < 0.005$
22 13 24 8 3 6 16 11 12 18 9 10 26 23 14 2
Queen ID


A
GP activity (U g-1 thorax)
0
3
6
9
12
a
a,b
a,b
b
W
G
SQ
Q
B
TRE activity (U g-1 thorax)
0
10
20
30
40
50
a
b
a,b
b
W
G
SQ
Q
C
HK activity (U g-1 thorax)
0
20
40
60
80
100
a
b
b
b
W
G
SQ
Q
Type of bumblebee
D
PGI activity (U g-1 thorax)
0
100
200
300
400
500
600
700
a,b
b
b
a
W
G
SQ
Q
Type of bumblebee