Heritability of Flight Energetics and its Associated Traits in the Bumblebee *Bombus impatiens*

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LIST OF ABREVIATIONS

FlightMR – Flight metabolic rate

GP – Glycogen phosphorylase

H^2 **–** Broad-sense heritability

h^2**-** Narrow-sense heritability

HK –Hexokinase

MS – Mean squares

OLS- Ordinary least squares

PGI - Phosphoglucoisomerase

RMR – Resting metabolic rate

SE – Standard error

τ – Intraclass correlation coefficient

TRE –Trehalase

$\mathbf{V_A}$ **–** Additive genetic variance

$\mathbf{V_D}$ **–** Dominance genetic variance

$\mathbf{V_E}$ **–** Environmental variance

$\mathbf{V_G}$ **–** Genetic variance

$\mathbf{V_I}$ **–** Interaction genetic variance

$\mathbf{V_P}$ **–** Phenotypic variance

$\dot{V}CO_2$ **-** CO_2 production rate

WBF – Wing beat frequency

WSA – Wing section area

ABSTRACT

Recent studies suggest a possible correlated evolution of wing morphology, wing beat frequency, muscle biochemistry and flight metabolic rate in bees. In order to investigate the degree to which natural selection can act on these traits, an estimation of heritability was required. Commercial and laboratory reared colonies from wild caught queens were used to estimate narrow-sense (h^2) and broad-sense (H^2) heritability of flight metabolic rate and its associated traits in the bumblebee *Bombus impatiens.* h^2 estimates obtained from parent-offspring regressions were not statistically significant. H^2 estimates were significant for morphological traits (body mass and wing morphology) as well as whole-animal traits (flight and resting metabolic rate, wing beat frequency) in both populations. We suggest that queens have a decrease in flight performance as a result of a trade-off between flight and fecundity, explaining the lack of significance in parent-offspring regressions.

RÉSUMÉ

De récentes études suggèrent une évolution corrélée de la morphologie des ailes, de la fréquence de battements d'ailes, des propriétés biochimiques musculaires et du taux métabolique de vol chez les abeilles. Afin d'en savoir plus sur comment la sélection naturelle peut agir sur ces caractères, il est nécessaire d'obtenir des estimés d'héritabilité. À l'aide de colonies commerciales et de colonies élevées en laboratoire à partir de reines sauvages, nous avons estimé l'héritabilité au sens strict (h^2) et au sens large (H^2) du métabolisme énergétique du vol et les caractères qui y sont associés chez le bourdon *Bombus impatiens*. Les estimés d'h^2 obtenus à partir de régressions parent-enfant n'étaient pas significativement différents de 0 pour tous les caractères. Les estimés d'H^2 étaient significatifs pour les caractères morphologiques (masse corporelle, surface des ailes) et les caractères physiologiques au niveau de l'animal entier (taux métabolique lors du vol, fréquence de battements d'ailes, taux métabolique de repos). Nous suggérons que les reines ont une diminution de leur capacité de vol à cause d'un compromis entre le vol et la fécondité, ce qui expliquerait les résultats non-significatifs des régressions parent-enfant.

1. GENERAL INTRODUCTION

1.1 Energy metabolism and locomotor performance

The capacity of acquiring and spending energy is of utmost importance for survival and reproductive success of an animal. Locomotor capacity in animals has a broad spectrum of variation, which relies on a diversity of traits found at multiple levels of organization, from whole-animal properties to physiological systems, morphology and biochemistry (Garland, 1988; Irschick and Garland, 2001). Many studies have tried to elucidate the mechanistic bases of interspecific differences in energy metabolism using the study of variations in locomotor performance. There is growing interest in the study of intraspecific variation in metabolic rate (Reidy et al., 2000; Lu et al., 2007; Skandalis and Darveau, 2012), given its implication in our understanding of evolution yielding interspecies differences. Differences among individuals of a same species (interindividual differences) can be considerable (Garland, 1984) and looking at individual variation instead of differences among populations or species can be advantageous in order to understand the evolution of metabolic properties.

Variations among species are the evolutionary result of natural selection acting on phenotypic variation among individuals. Individual variation is linked to the study of microevolutionary change caused by selection on differences in phenotypes that have a genetic basis (Lande and Arnold, 1983). Microevolutionary changes occur in time scales that can be potentially observable by biologists (Hayes and Jenkins, 1997). When selection

operates on traits for which heritable genetic variation exists, evolutionary change across generations may occur. To investigate whether a trait responds to natural selection, three different issues must be considered: the performance trait must show variation amongst individuals that is relatively stable over time (their performance should be repeatable); it must be shown to contribute to differential fitness among individuals; and it must be heritable (Endler, 1986).

1.2 Repeatability

Repeatability is a measurement of the stability of an observable trait within individuals, relative to differences in that trait among individuals (Van Berkum et al., 1989). Greater between- than within- individual variation indicates that the trait is consistent within individuals, and in turn, would be able to respond to selection (Artacho and Nespolo, 2009). Significant repeatability in a trait suggests that the trait may have a genetic basis, and values of repeatability are often thought to estimate an upper limit to heritability (Falconer and Mackay, 1996; but see Dohm, 2002; Naya, 2010).

Repeatability of metabolic rate has received a lot of attention in recent years and studies have looked at a variety of models ranging from mammals (Friedman et al., 1992; Chappell et al. 1995; Fournier and Thomas, 1999; Labocha et al., 2004; Rezende et al., 2005), reptiles (Garland and Bennett, 1990), birds (Bech et al., 1999; Fynh et al., 2001; Horak et al., 2002; Rønning et al., 2005), fish (McCarthy, 2000; Virani and Rees, 2000) and insects (Chappell and Rogowitz, 2000; Nespolo et al., 2003; Terblanche et al., 2004).

Nespolo and Franco (2007) conducted a meta-analysis combining results from over 45 studies looking at the repeatability of resting and active metabolic rate in a wide range of animals. Their results show that metabolic rate is repeatable in most of the species investigated. Nevertheless, few studies have looked at flight metabolic rate in insects, the subject of the present study.

Since flying insects have some of the highest metabolic rates in the animal kingdom (Sacktor, 1976), they are good models for the study of energy metabolism and its evolution. In flight muscles, respiration and energy production may increase several hundredfold during the transition from rest to flight (Candy et al., 1997). Among flying insects, some rely heavily on their flight performance for their daily activity. Some species of bees are central place foragers, which means that they have to return to their nest several times a day carrying pollen and nectar. Flight performance is therefore crucial to their success and survival. Bees use carbohydrates as their main source of energy (Suarez et al., 2005), which simplifies the study of their metabolic pathways. Moreover, about 90% of metabolic rate during hovering flight is caused by oxygen consumption from flight muscle tissues (Suarez, 2000).

Darveau et al. (in press), studied the repeatability of flight energetics in *Bombus impatiens* workers. They wanted to answer the question: do individuals that flap their wings faster and consume more oxygen per unit time always do so when they fly? In this study, repeatability in hovering flight metabolic rate (measured as the rate of CO_2 production per

hour) and wing beat frequency (in wing beats per second) were high, which suggests strong genetic basis (Darveau et al, in press).

1.3 Metabolic Scaling

Variation in metabolic rate shows a strong dependence on body mass. This relationship is expressed as a power function and it has been widely accepted that the scaling exponent is 3/4, or follows what has been named the '3/4 power law' (Peters, 1983; Savage et al., 2004) . However, this law has been the subject of debate and recent studies show that the scaling exponent might not always be 3/4 (Glazier, 2005; Packard and Birchard, 2008). As a general rule, metabolic rate scales positively with body mass and follows an allometric relationship (the slope is different from one). This has been observed in vertebrates, such as birds, fish and mammals (Bennett and Harvey, 1987; Bishop, 1999; Clarke and Johnston, 1999; White and Seymour 2005) as well as invertebrates such as arthropods (Darveau et al., 2005a; Terblanche and Anderson, 2010; DeVries et al., 2013).

Although a large portion of intraspecific differences in metabolic rate can be explained by variations in body mass in *B. impatiens*, variation in flight metabolic rate can be as high as three-fold for same size individuals in similar environmental conditions (Skandalis and Darveau, 2012; Darveau et al. in press). In the process of elucidating the physiological bases of such variation, these studies have demonstrated correlations between wing morphology, wing beat frequency and hovering flight metabolic rate. Bees with larger wings for their body size exhibit lower wing beat frequencies and therefore lower hovering flight metabolic rate. In addition to morphological traits, muscle biochemical properties seem

to be linked with metabolic rate. Enzymes involved in glycolysis (glycogen phosphorylase, trehalase, hexokinase, phosphoglucoisomerase, pyruvate kinase) and in Kreb's cycle (citrate synthase) have activities that are positively correlated with flight metabolic rate in bees (Skandalis and Darveau, 2012; Darveau et al., in press). Hexokinase and trehalase activities were associated with higher hovering flight metabolic rate in both studies. These correlations between wing morphology, wing beat frequency, muscle biochemical properties and flight metabolic rate found within species have also been shown among species of bees (Darveau et al., 2005a, Darveau et al., 2005b) suggesting a strong functional association among these traits that are possibly under selection.

Although previous studies suggest that flight performance and its associated traits have a strong genetic basis in *B. impatiens*, the amount of variation in a population that is caused by genetic differences remains unknown. The goal of this study was to further investigate the genetic bases of the flight phenotype using heritability estimates in commercial and wild bumblebee colonies.

2. INTRODUCTION

A phenotype is any observable trait of an organism, therefore the phenotypic value of an individual is the measured value of that trait. The phenotypic value can be influenced by the individual's genotype and the environment. The genotype is the particular combination of genes of an individual, and the environment includes all of the non-genetic factors that influence phenotypic value. In order to estimate the relative importance of the determinants of the phenotype, it is necessary to partition the amount of variation (variance) attributable to the genetics or the environment. The total variance is the sum of the components:

$$V_P = V_G + V_E \quad (1)$$

$$V_P = V_A + V_D + V_I + V_E \quad (2)$$

Where V_p is the total phenotypic variance, V_G is the genetic variance and V_E the environmental variance. The genetic variance (V_G) can be partitioned in additive variance (V_A), dominance variance (V_D) and interaction variance (V_I). The additive variance is a measure of how an individual's genetic makeup contributes to the phenotypic value of the next generation, or the cumulated effects of genes. The dominance variance is the variation caused by an interaction between alleles at a single locus, such as dominance. The interaction variance or epistatic variance is the interaction between genes at different loci that act on the same trait.

Heritability is the fraction of the phenotypic variation of a trait in a population that is due to genetic differences among individuals (Freeman and Herron, 2007). There are, however, two different meanings of heritability. Broad-sense heritability (H^2) is the ratio of genotypic variance (V_G) on total variance (V_P) and refers to the degree to which individuals phenotypes are determined by their genotype. Narrow-sense heritability (h^2) is the ratio of additive genetic variance (V_A) on the total variance (V_P) or phenotypic values and determines the extent to which phenotypes are determined by the genes transmitted from the parents (Falconer and Mackay, 1996).

Traditionally, narrow-sense heritability estimates were obtained from parent-offspring regressions. This method consists in measuring a trait in the parents and subsequently in the offspring, using the slope of the regression of offspring on parent value as the heritability estimate (Falconer and Mackay, 1996). In recent years, the study of evolutionary quantitative genetics has moved to the animal model using large pedigrees of known parents and offspring to estimate quantitative genetic parameters in populations (Knott et al., 1995; Äkesson et al., 2008). A recent study compared the two methods and found no overall differences in estimates and associated standard error between the parent-offspring regression and the animal model (Äkesson et al., 2008), suggesting that both methods are equivalent with regards to estimation of heritability. Broad-sense heritability estimates can be obtained using full-sib analyses, which analyze the amount of variance both within and among families. The idea is that if the offspring of one family are more similar to one another than offspring of other families for a trait, then this trait is heritable. This method uses only offspring values, and therefore the parents are not included in the analyses. This estimate, however, includes other possible effects such as dominance or epistatic genetic

variance as well as environmental variance associated with rearing in a common environment and maternal effects (Falconer and Mackay, 1996).

Ideally, estimates of natural heritability should be obtained from relatives in the wild, as it has been argued that laboratory studies may overestimate heritability. This would be based on the assumption that environmental variation in a laboratory setting is much smaller than what occurs under natural conditions (Weisenberg and Roff, 1996). Since heritability represents a ratio of genetic variation on total or phenotypic variation, lower environmental variance would increase heritability values. A challenge associated with measuring field heritabilities in animals is the capture or tagging of parents and their offspring, especially in species that have large dispersal rates. Riska et al. (1989) describe a different method for obtaining natural heritabilities using a regression of the trait values of lab-reared offspring on their wild-caught parents. This method is useful, because it allows an estimation of heritability which would be closer to "natural" heritability values, without the challenge of having to capture the offspring in the wild.

Heritability of locomotor performance has been investigated in a range of species. Several studies have examined locomotor ability in cows and horses using leg conformation or biokinematic variables such as hoof height or leg swing duration (Boelling and Pollott, 1998; Onyiro and Brotherstone, 2008; Valera et al., 2008). Traits such as running and swimming speed, endurance and aerobic capacity have been found to be heritable in mice and rats (Dohm et al., 1996; Koch et al., 1998; Swallow et al., 1998; Lightfoot et al., 2001). High values of broad-sense heritability were found in garter snakes for $\dot{V}O_{2MAX}$ (H^2=0.89,

Garland and Bennett, 1990) and in the fence lizard, with a range of 0.33-0.36 for sprint speed (Tsuji et al., 1989). Blumstein et al. (2010) found that heritability of locomotor ability is low, but significant in marmots.

In insects, different components of flight performance, such as flight distance and flight duration have been shown to be heritable (with both narrow and broad-sense heritability estimates) in a range of species (Caldwell and Hegman, 1969; Gu and Danthanaranaya, 1992; Han and Gatehouse, 1993; Gu and Barker, 1995; Schumacher et al., 1997; Han et al., 2009; Tanaka, 2009). Some studies have generated heritability estimates for flight associated traits in insects, such as wing morphology, wing shape and wing beat frequency (Curtsinger and Laurie-Ahlberg, 1981; Van Dyck et al., 1998; Bitner-Mathé and Klaczko, 1999c; Begin and Roff, 2002; Moraes and Sene 2004). In honeybees, various morphological traits have been shown to be heritable, such as wing length and width as well as other body size indicators (Oldroyd et al. 1991, Poklukar and Nezic, 1994). Heritability of body size parameters has also been studied in other Hymenopterans (Tepedino et al., 1984; Diniz and Pingnata,1994). However, most studies performed on honeybees focus on traits related to either honey production (Milne, 1985; Bienefeld and Pirchner, 1990; Jevtić et al., 2012; Zakour et al., 2012), brood parameters (Le Conte et al., 1994) or resistance to parasites (Boecking et al., 2000; Stanimirović, 2010). To date very little is known about the heritability of flight metabolic rate and its associated traits in insects.

3. OBJECTIVES AND STUDY QUESTIONS

In the present study, our primary interest was to estimate both narrow-sense and broad-sense heritability of flight energy metabolism and its associated phenotypic traits at different levels of organization.

Previous work provides useful information on how to characterize the bumblebee flight phenotype at different levels of organization. However, these studies were performed mostly on workers and no information is available for bumblebee queens. Therefore, we first characterized variation in the flight phenotype by assessing scaling of flight variables in workers and in queens. We subsequently examined the relationships among some flight performance traits (wing section area, wing beat frequency, flight metabolic rate). Then, since we had information on the repeatability of body mass, metabolic rate and wing beat frequency in *B. impatiens* workers (Darveau et al., in press), we further tested this assumption in queens.

Based on previous work (Darveau et al., 2005a and b; Skandalis and Darveau, 2012; Darveau et al., in press), we measured traits that are correlated and seem to adequately characterize hovering flight phenotype in the bumblebee *B. impatiens*. At the whole animal level, we measured flight metabolic rate (FlightMR), resting metabolic rate (RMR) and wing beat frequencies (WBF). At the morphological level, we measured body mass and measured a wing section area (WSA) as a proxy of total wing surface area. Finally, at the cellular metabolic level, we measured the activity of the enzymes glycogen phosphorylase (GP),

trehalase (TRE), hexokinase (HK) and phosphoglucoisomerase (PGI). To contrast natural and laboratory heritabilities, we used both commercial colonies and colonies reared in the laboratory from wild caught queens. This also allowed us to compare flight phenotypes of the two different populations (commercial and wild *B. impatiens*) in order to detect possible genetic differences in the traits of interest.

4. MATERIALS AND METHODS

4.1 Animals and holding conditions

Thirty colonies of *Bombus impatiens* were generously provided by a commercial supplier (Biobest Canada Ltd, Leamington, ON, Canada), and 27 colonies were obtained from laboratory rearing of queens collected in the wild from 5 distinct locations in the Gatineau (Quebec, Canada) region from mid-May to mid-June 2011. Commercial colonies were kept in the suppliers housing boxes in a room maintained at 25˚C with a 12L:12D photoperiod. Colonies were provided with unlyophilized pollen and sucrose solution *ad libitum* and acclimated to these laboratory conditions for 1 week after arrival. Whole-animal measurements were conducted within the week following the acclimation period. All individuals were then dissected and stored at -80˚C for morphological and cellular measurements.

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 bee 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.

4.2 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 flight success of queens over the first week of acclimation. We further documented time dependence of flight success and flight measurements in the wild caught queens, by performing 4 series of flight measurements over the course of the experiment. 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) 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. A 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 ($\dot{V}CO_2$ values) were measured using a FoxBox flow-through respirometry system (SSI). The CO_2 analyzer was calibrated daily using a two point

calibration using Nitrogen (0% CO_2) and 402 ppm CO_2 span gas. All flight measurements were taken at room temperature. Bees were flown in a 1 L glass flask equipped with a sidearm at its base. Air was drawn from the environment, dried using Dessicant Media (Permapure, Toms Rivers, NJ, USA) and pushed in the respirometry system at a rate of 1 L min^{-1}. Bees were flown until a steady state flight and CO_2 production rate were observed for more than 30 seconds. Flights of 5-10 min were sufficient to obtain these conditions. If needed, we facilitated flight with light shaking of the flask when bees attempted to land.

Metabolic rate was calculated based on CO_2 production rates recorded and analysed using the data acquisition and analysis software Expedata (SSI). CO_2 baselines were recorded before and after bees were inserted in the flask. We assumed that *B. impatiens* powered flight using exclusively carbohydrates as documented in many other bee species (Suarez et al. 2005), which appears valid as this species flight muscle permeabilized fibers do not oxidize palmitoyl-carnitine (C.-A. Darveau, unpublished data).

The equation used to calculate $\dot{V}CO_2$ is as follows: $\dot{V}CO_2$ = Flow rate x ($FECO_2$ – $FICO_2$), where $FECO_2$ is the fraction of CO_2 in expired air and $FICO_2$ is the fraction of CO_2 in inspired air (baseline).

During FlightMR measurements, wing beat frequency was measured using an optical detector (Moore Scientific) placed under the respirometry chamber. The detector was linked to a portable computer and the signal was recorded and analyzed using the software Trex 2.0

Transient Waveform Recorder (Moore Scientific). We used a mean value of 10 measurements for each individual.

4.3 Resting Metabolic Rate

Metabolic rate at rest (RMR) was measured in commercial colonies only. Limited space in the respirometer did not allow for measurements in wild colonies. 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 at a rate of 60-120 ml/min into 8 respirometry chambers. 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 7 chambers and 1 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 indicator of a resting state.

4.4 Morphological measurements

Upon completion of whole-animal measurements, individuals were anesthetised 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 using an analytical balance (Mettler-Toledo). 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, but slightly allometric (b=0.94, r^2=0.949, P < 0.001, n=461).

4.5 Cellular Metabolic Measurements

Thoraxes were minced with scissors and homogenized in 19-volumes of ice-cold buffer. All further manipulations were carried on crushed ice. The homogenization buffer used consisted of 25 mM Tris-potassium phosphate pH 7.76 at 5˚C, 2 mM ethylene diaminetetraacetic acid (EDTA), 5 mM dithiothreitol (DTT) and 0.5% (v/v) Triton X-100. Minced thoraxes were homogenized three times for 10 seconds at 10 000 rpm with 30 seconds cooling intervals using an Omni-prep multi-sample homogenizer (Omni International inc., Kennesaw, GA, USA). Homogenates were then sonicated three times for 10 seconds with 30 seconds cooling intervals using a sonicator (Vibra-Cell VC750, Sonics, Newtown, CT, USA). Finally, homogenates were centrifuged for 5 min at 5000 rpm at 4˚C, and supernatants were used for assays.

Enzyme activities were measured in triplicates using a Sinergy 2 Multi-Detection Microplate Reader (Biotek Instruments Inc., Winooski, VT, USA) maintained at 37˚C. Glycogen phosphorylase (GP), trehalase (TR), hexokinase (HK) and phosphoglucoisomerase (PGI) reactions were monitored using the rate of appearance of nicotinamide adenine dinucleotide phosphate (NAPDH) at 340 nm using 6.22 as a millimolar extinction coefficient (ε). In cases where control rates were different from zero, we subtracted them from rates obtained with all substrates present . In preliminary experiments, reagents concentrations were varied to ensure that the highest activities were achieved. Enzyme assays were also conducted at optimal pH values (see Skandalis et al., 2011). All enzyme activities were measured in triplicate and the data presented for each individual represents the mean of these

3 measures. In cases where too much variability was observed, best duplicates were used. Enzyme activities are presented as µmoles of product per min. per gram of thorax or U g^{-1} thorax.

Assay conditions were as follows: **GP**: 100 mM l^{-1} Potassium phosphate pH 7.1 at 37˚C, 10 mM l^{-1} $MgCl_2$, 4 mg ml^{-1}glycogen (omitted for control), 0.75 mM l^{-1} $NADP^+$, 4 µmol l^{-1} glucose 1,6-biphosphate, 2 mM l^{-1} AMP, 5 U ml^{-1}phosphoglucomutase and 5 U ml^{-1} glucose-6-phosphate dehydrogenase. **TR**: 50 mM l^{-1} Potassium phosphate pH 6.6 at 37˚C, 1.1 mM l^{-1} $MgCl_2$, 10 mM l^{-1}trehalose (omitted for control), 0.75 mM l^{-1} $NADP^+$, 1.1 mM l^{-1} ATP, 5 U ml^{-1} hexokinase and 5 U ml^{-1} glucose-6-phosphate dehydrogenase. **HK**: 100 mM l^{-1}tris-imidazole pH 8.1 at 37˚C, 100 mM l^{-1}KCl, 10 mM l^{-1} $MgCl_2$, 5 mM l^{-1}D-glucose (omitted for control), 1 mM l^{-1} $NADP^+$, 5 mM l^{-1} ATP and 5 U ml^{-1} glucose-6-phosphate dehydrogenase. **PGI**: 50 mM l^{-1}Tris-imidazole pH 8.1 at 37˚C, 5 mM l^{-1}KCl, 10 mM l^{-1} $MgCl_2$, 16 mM l^{-1} fructose-6-phosphate (omitted for control), 0.75 mM l^{-1} NADP and 5 U ml^{-1} glucose-6-phosphate dehydrogenase.

4.6 Statistical Analyses and Heritability Estimates

4.6.1 Phenotypic values

In bumblebee workers, interindividual variation is strongly determined by variation in body size. We therefore examined the scaling exponent (*b*) of each variable on body mass using ordinary least squares (OLS) regression of log transformed dependent variable on log transformed body mass. We performed ANCOVAs to test for differences in slopes and intercepts between commercial and wild-caught populations using body mass as a covariate. When no significant differences in slope and intercept were detected, we pooled data from both populations in the regression analyses. When no significant interaction was detected, it was removed from the model.

We performed a Pearson's correlation analysis in order to assess the relationship between residuals of some flight traits (WSA, WBF and Flight MR) from the body mass relationship. Data presented in workers only.

In order to test differences in enzyme activities between groups (workers, gynes, spring queens and queens) we used ANCOVAs with body mass as a covariate. Tukey post-hoc comparisons were performed to test differences between groups

.

4.6.2 Repeatability and Phenotypic Variation in Queens

In order to evaluate the phenotypic variation of performance traits (FlightMR, WBF) in queens, we performed an ANCOVA with body mass as a covariate and colony state as a factor. We performed measurements at 4 different states: before the establishment of the colony, one week after the emergence of the first worker, when the colony reached 30-40 workers and one week after the latter. The intraclass correlation coefficient (τ) was used as an estimate of repeatability in queens (see equation 6).

4.6.3 Heritability Estimates

A total of 27 commercial and 18 wild colonies were used to estimate narrow-sense heritability using the parent-offspring method. The sample size was reduced in wild colonies, as some of the wild queens either died or were no longer able to fly when measurements were taken. WSA, FlightMR, WBF and RMR scaled with body mass. We therefore corrected for size using the residuals of the regressions of these traits on body mass.

Using parent-offspring regression to assess heritability, variance of traits should be equivalent between parent and offspring generations (Lynch and Walsh, 1998). Therefore, we standardized values (such that mean=0 and variance=1) before regression analyses were performed. Standardization was performed by subtracting mean values of the trait and dividing by the standard deviation. Standardized mid-offspring values of each trait were regressed on those of their mother using ordinary least squares. Since regressions were performed using a single parent on offspring regression, the slope of the regression was

multiplied by 2 to give a value of narrow sense heritability (h^2) and the standard error of the regression was multiplied by 2 to give a standard error of h^2 (see Falconer and Mackay, 1996). A separate regression was performed on each population. Whole-animal measurements (FlightMR, RMR, WBF) were performed on queens when the colony was at around 40-70 workers. In order to contrast for possible phenotype changes in queens we also performed a parent-offspring regression using wild queen measurements taken in the field before the establishment of their colonies.

Broad-sense heritability estimates were obtained using components of variances from a one-way full-sib ANOVA with colony (family) as the main effect (Falconer and Mackay, 1996). We included mass as a cofactor when it was significant (FlightMR, RMR, WBF, WSA).

We used the following equation to calculate variance components:

$$\mathrm{Var}(f) = \frac{\mathrm{MS}_f - \mathrm{MS}_w}{n} \quad (3)$$

$$\mathrm{Var}(w) = \mathrm{MS}_w \quad (4)$$

$$\mathrm{Var}(z) = \mathrm{Var}(f) + \mathrm{Var}(w) \quad (5)$$

Where, Var(*f*) is the between-family variance, Var(*w*) is the within-family variance and Var(*z*) is the total variance. MS is the mean square obtained as a result of the ANOVA. From these estimates, we can calculate the intra-class correlation coefficient (τ_{FS}):

$$\tau_{FS} = \frac{\mathrm{Var}(f)}{\mathrm{Var}(z)} \quad (6)$$

We can then calculate heritability (H^2), which is double the intra-class correlation coefficient.

Standard error (SE) was calculated as follows:

$$\mathrm{SE}(H^2) \cong 2(1 - \tau_{\mathrm{FS}})[1 + (n-1)\tau_{\mathrm{FS}}]\sqrt{2/[Nn(n-1)]} \quad (7)$$

Where N is the number of full-sib families, and n is the number of offspring per family.

Shapiro-Wilk tests were performed to assess normality of data. Levene test was used to test for equality of variance. Statistical analyses were performed using the software SYSTAT 13.0 (Chicago, USA). For all analyses, $\alpha = 0.05$.

5. RESULTS

5.1 Phenotypic values

5.1.1 Scaling of flight performance traits in workers and queens

Since variation between individuals is strongly determined by body mass, we performed regression analyses to determine the scaling exponent (*b*) of the different variables on body mass (see Table 5.2). Prior to these analyses, we tested for differences in slopes and intercepts between populations using ANCOVAs with body mass as a covariate (Table 5.1). When no difference was detected, we pooled data from both groups in the analysis. The slope and intercept of the relationship between body mass and FlightMR did not differ between populations. We therefore performed a regression analysis on pooled data for this trait in workers (Figure 5.1 and Table 5.2). WSA scaled isometrically with body mass in commercial and wild workers, based on a b=2/3 exponent. FlightMR and RMR scaled identically in workers (see Table 5.2) and body mass explained 57 and 50% of their variance, respectively. WBF scaled negatively with body mass with similar exponents in both populations. HK scaled negatively with body mass, although the relationship was only significant in commercial bees (see Table 5.2 and Figure 5.3). PGI activity increased with body mass in wild colonies, however the explained variance was only 7%. The slope of the relationship between body mass and TRE was significantly different in the two populations, but was not significant in either of them.

In Figure 5.1 A and D, we observe that queens fall on the curve predicted by the relationship in workers for WSA and RMR. However, for FlightMR, spring queens fall on the curve, whereas older queens fall under the curve predicted by workers.

Despite the small sample sizes, we also assessed the effect of body mass on each variable in queens using OLS regressions (see Table 5.2). Since the relationships between body mass and WSA were similar in both populations, we analyzed data from commercial and wild queens together. WSA scaled positively with body mass with an exponent of 0.20±0.06. However, this explained only 14% of the variance, which is lower than what was observed in workers (see Table 5.2). RMR, which was only measured in commercial queens, also scaled with body mass, with an exponent of 0.49±0.14. The relationship between FlightMR and body mass was different between both populations and significant in wild queens only (Tables 5.1 and 5.2). WBF did not scale with body mass in queens in either population. At the cellular metabolic level, hexokinase activity decreased with body mass in queens (Table 5.2).

5.1.2 Comparison of commercial and wild bees

Commercial workers were slightly smaller and had higher WBF than wild ones after accounting for body mass (Table 5.1). However, these differences are very small (less than 3%) and significance could be detected due to the large sample size (n=822). Although WSA was almost identical between both populations (Table 5.1), commercial bees have slightly larger wings relative to their body size (Table 5.1). Wild queens were slightly larger than

commercial ones and had higher FlightMR relative to their size. In addition, they had lower WBF than their commercial counterparts.

When considering mid-offspring values, most enzyme activities are higher in wild colonies (see Table 5.1 and Figure 5.3). For instance, in workers, TRE mean activity was almost 20% higher in wild bees than in commercial ones, and HK and PGI activity were 15% and 14% higher in wild colonies respectively (Table 5.1). GP activity was 11% higher in wild colonies. In queens, none of the enzymes scaled with body mass in either population. In addition, there were no significant enzyme activity differences between wild and commercial queens (see Table 5.1).

5.1.3 Correlation analyses of phenotypic traits

In workers, results of Pearson's correlation (Figure 5.2) show that WSA residuals from the body mass relationship are negatively correlated with WBF ($r=-0.39$, $p<0.001$, $n=396$). This means that bees with larger wings for their body size beat their wings slower than bees with smaller wings. WBF residuals from the body mass relationship were also positively correlated with FlightMR residuals ($R=0.43$, $p<0.001$, $n=820$), indicating that bees with higher WBF for their size also have higher FlightMR than bees with a similar body mass. Contrary to what was observed in workers, there were no significant correlations between WSA, WBF and FlightMR residuals from the body mass relationship in queens.

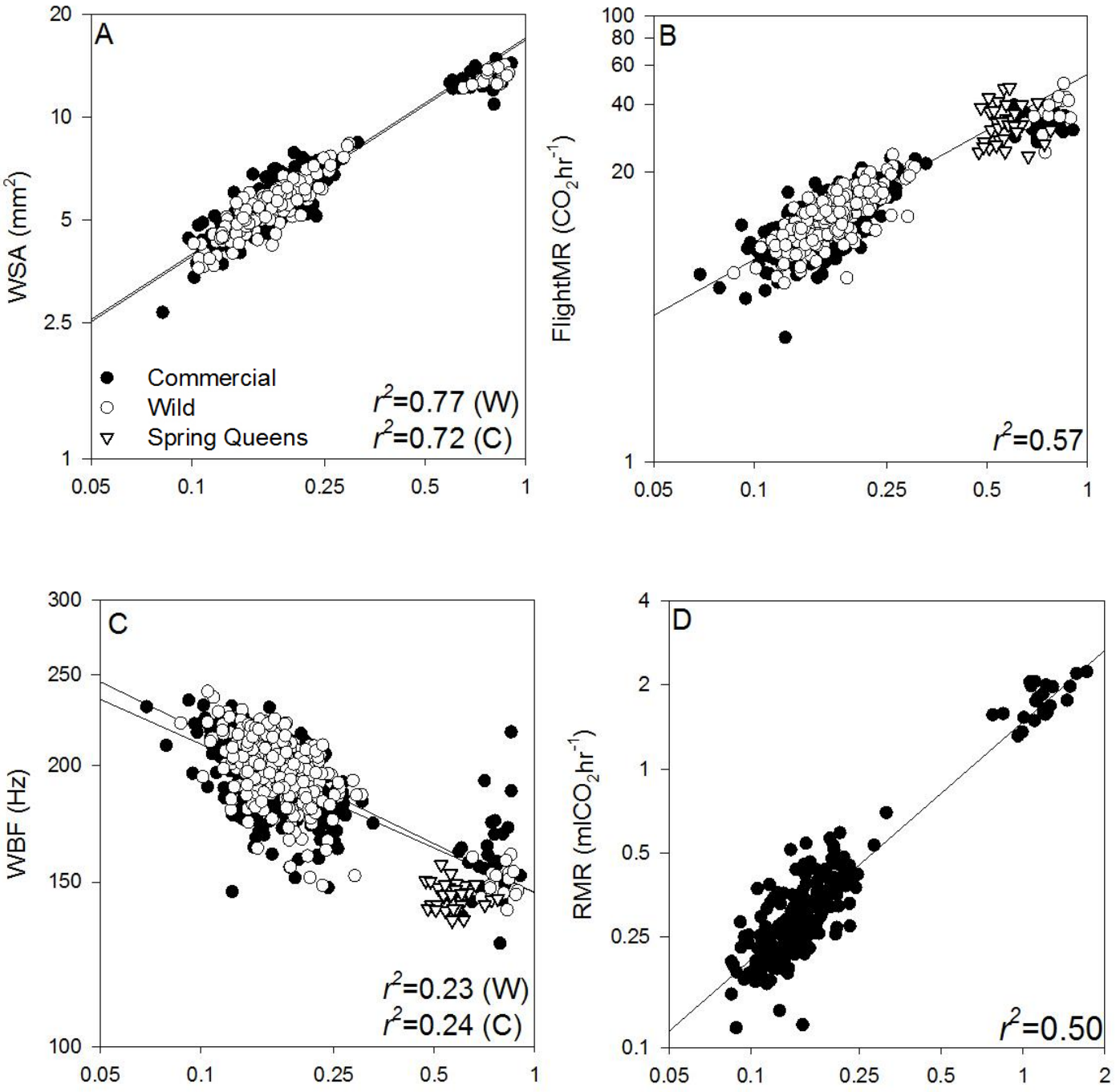

A
WSA (mm²)
20
10
5
2.5
1
0.05
0.1
0.25
0.5
1
Commercial
Wild
Spring Queens
r²=0.77 (W)
r²=0.72 (C)
B
FlightMR (CO₂hr⁻¹)
100
80
60
40
20
1
r²=0.57
C
WBF (Hz)
300
250
200
150
100
r²=0.23 (W)
r²=0.24 (C)
D
RMR (mlCO₂hr⁻¹)
4
2
1
0.5
0.25
0.1
2
r²=0.50
Body Mass (g)

Figure 5.1 Relationship between body mass (g) and (A) WSA (mm^2), (B) flight metabolic rate (FlightMR) (ml CO_2 hr^{-1}), (C) wing beat frequency (Hz) and (D) resting metabolic rate (ml CO_2 hr^{-1}) in commercial (C, closed circles) and wild (W, open circles) *B. impatiens* workers, spring queens (triangles) and queens. Regression data presented for workers only. All relationships were significant ($p<0.001$)

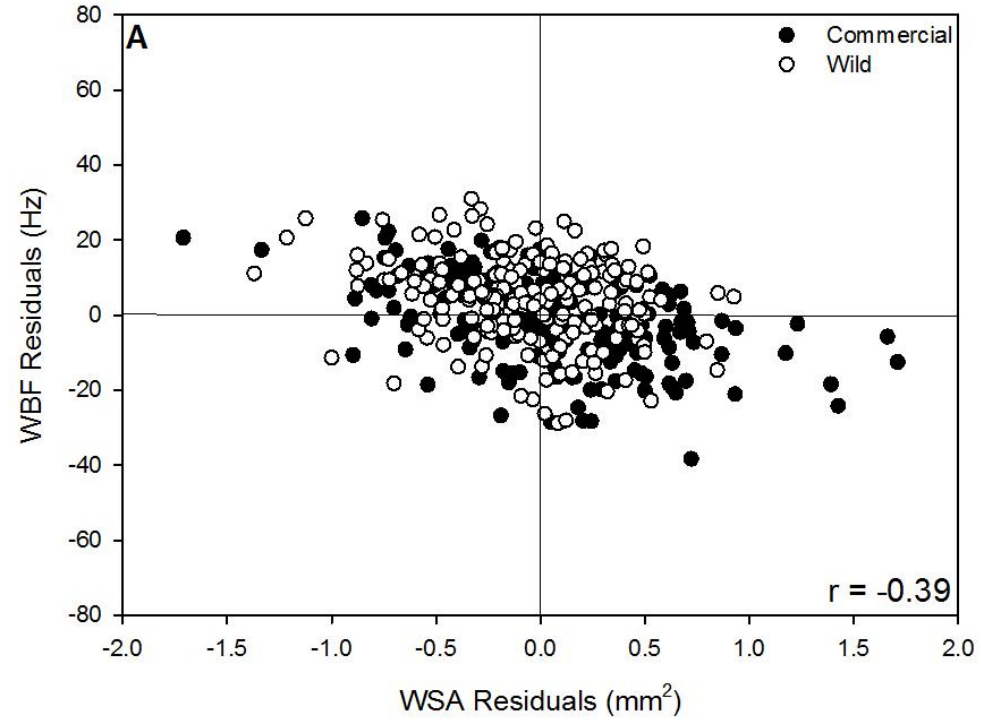
Table 5.1 Mean values and standard error of the mean (SE) of wing section area (WSA), flight metabolic rate (FlightMR), wing beat frequency (WBF), glycogen phosphorylase (GP), trehalase (TRE), hexokinase (HK) and phosphoglucoisomerase (PGI) activities presented for workers (offspring generation) and queens (parent generation) of commercial and wild *B. impatiens*.

	Workers				Queens			
Trait	Commercial	Wild	Analysis of variance[a]		Commercial	Wild	Analysis of variance[a]	
	Mean (SE)	Mean (SE)	F	*P*	Mean (SE)	Mean (SE)	F	*P*
Mass (g)	0.171 (0.003)	0.176 (0.005)	5.11	0.024	0.741 (0.016)	0.792 (0.016)	4.41	0.042
WSA (mm^2)	5.61 (0.07)	5.59 (0.06)	4.25	0.040	13.04 (0.16)	13.05 (0.14)	0.51	0.479
FlightMR (ml CO_2 hr^{-1})	12.61 (0.18)	13.20 (0.15)	3.46	0.063	33.57 (0.60)	37.23 (1.60)	12.58	0.001
RMR (ml CO_2 hr^{-1})	0.31 (0.01)	-	-	-	1.17 (0.03)	-	-	-
WBF (Hz)	194.62 (0.66)	198.63 (0.75)	32.96	<0.001	162 (3)	150 (1)	6.16	0.017
GP (U g^{-1})	8.42 (0.18)	9.30 (0.20)	9.63	0.020	7.75 (0.37)	8.803 (0.47)	1.23	0.273
TRE (U g^{-1})	35.02 (0.62)	41.95 (0.72)	9.50	0.002	22.03 (0.90)	24.22 (1.00)	0.94	0.337
HK (U g^{-1})	73.68 (0.78)	84.54 (0.67)	114.53	<0.001	53.45 (2.39)	51.42 (1.09)	0.96	0.331
PGI (U g^{-1})	405.38 (6.89)	463.21 (7.25)	31.86	<0.001	450.48 (14.65)	458.24 (18.21)	1.56	0.218

[a] _Results of ANOVA (for body mass) and ANCOVAs accounting for body mass testing for differences between populations. Analyses performed on log10 transformed data. When non-significant, interactions were removed from model.

Table 5.2 Scaling of wing section area (WSA), flight metabolic rate (FlightMR), wing beat frequency (WBF), glycogen phosphorylase (GP), trehalase (TRE), hexokinase (HK) and phosphoglucoisomerase (PGI) activities on body mass in *B. impatiens* workers and queens of commercial (C) and wild (W) colonies. The scaling exponent, *b*, was determined by OLS regression of log transformed body mass and log transformed dependent variable. When no difference in slope and intercept were detected, we pooled data from both groups for analyses.

	Workers				Queens			
Trait	*b* (SE)	r^2	*P*	N	*B* (SE)	r^2	*P*	N
WSA (mm^2)	(W) 0.63 (0.02)	0.77	<0.001	268	0.20 (0.06)	0.14	0.011	44
	(C) 0.63 (0.03)	0.72	<0.001	194				
FlightMR (ml CO_2 hr^{-1})	0.83 (0.03)	0.57	<0.001	822	(W) 1.07 (0.43)	0.31	0.026	16
					(C) -0.04 (0.16)	0.002	0.812	27
RMR (ml CO_2 hr^{-1})	(C) 0.85 (0.06)	0.50	<0.001	195	(C) 0.49 (0.14)	0.38	<0.001	24
WBF (Hz)	(W) -0.17 (0.02)	0.23	<0.001	351	(W) -0.05 (0.11)	0.01	0.677	16
	(C) -0.16 (0.01)	0.24	<0.001	469	(C) 0.25 (0.18)	0.07	0.186	27
GP (U g^{-1})	(W) 0.40 (0.28)	0.01	0.153	144	0.28 (0.24)	0.03	0.255	52
	(C) 0.13 (0.23)	0.002	0.573	162				
TRE (U g^{-1})	(W) 0.35 (0.20)	0.02	0.093	144	0.001 (0.19)	0.00	0.998	52
	(C) -0.25 (0.17)	0.01	0.144	162				
HK (U g^{-1})	(W) -0.17 (0.10)	0.02	0.106	144	-0.28 (0.13)	0.08	0.044	52
	(C) -0.37 (0.11)	0.07	0.001	162				
PGI (U g^{-1})	(W) 0.45 (0.20)	0.04	0.023	144	0.19 (0.18)	0.02	0.296	52
	(C) 0.05 (0.17)	0.00	0.796	161				


A
Commercial
Wild
WBF Residuals (Hz)
WSA Residuals (mm^2)
r = -0.39
80
60
40
20
0
-20
-40
-60
-80
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0

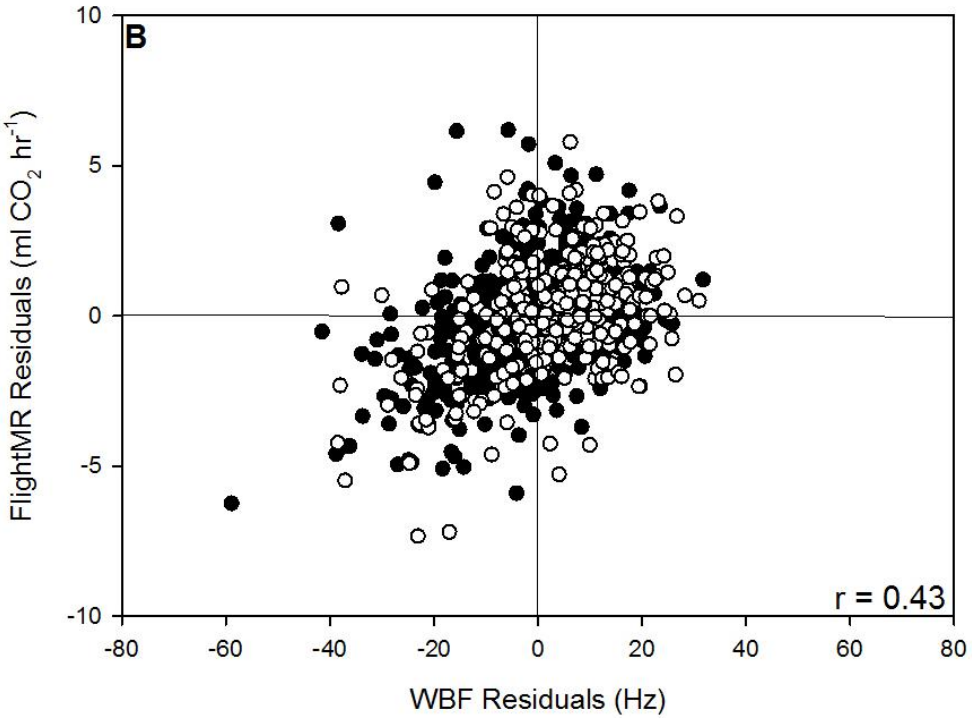

B
FlightMR Residuals (ml CO_2 hr^{-1})
WBF Residuals (Hz)
r = 0.43
10
5
0
-5
-10
-80
-60
-40
-20
0
20
40
60
80

Figure 5.2 Correlation between (A) wing section area (WSA) and wing beat frequency (WBF) residuals from the body mass relationship (n=396) and (B) between WBF and flight metabolic rate (FlightMR) residuals (n=820) from the body mass relationship in commercial (C, closed circles) and wild (W, open circles) *B. impatiens* workers. All correlations were significant ($p<0.001$).

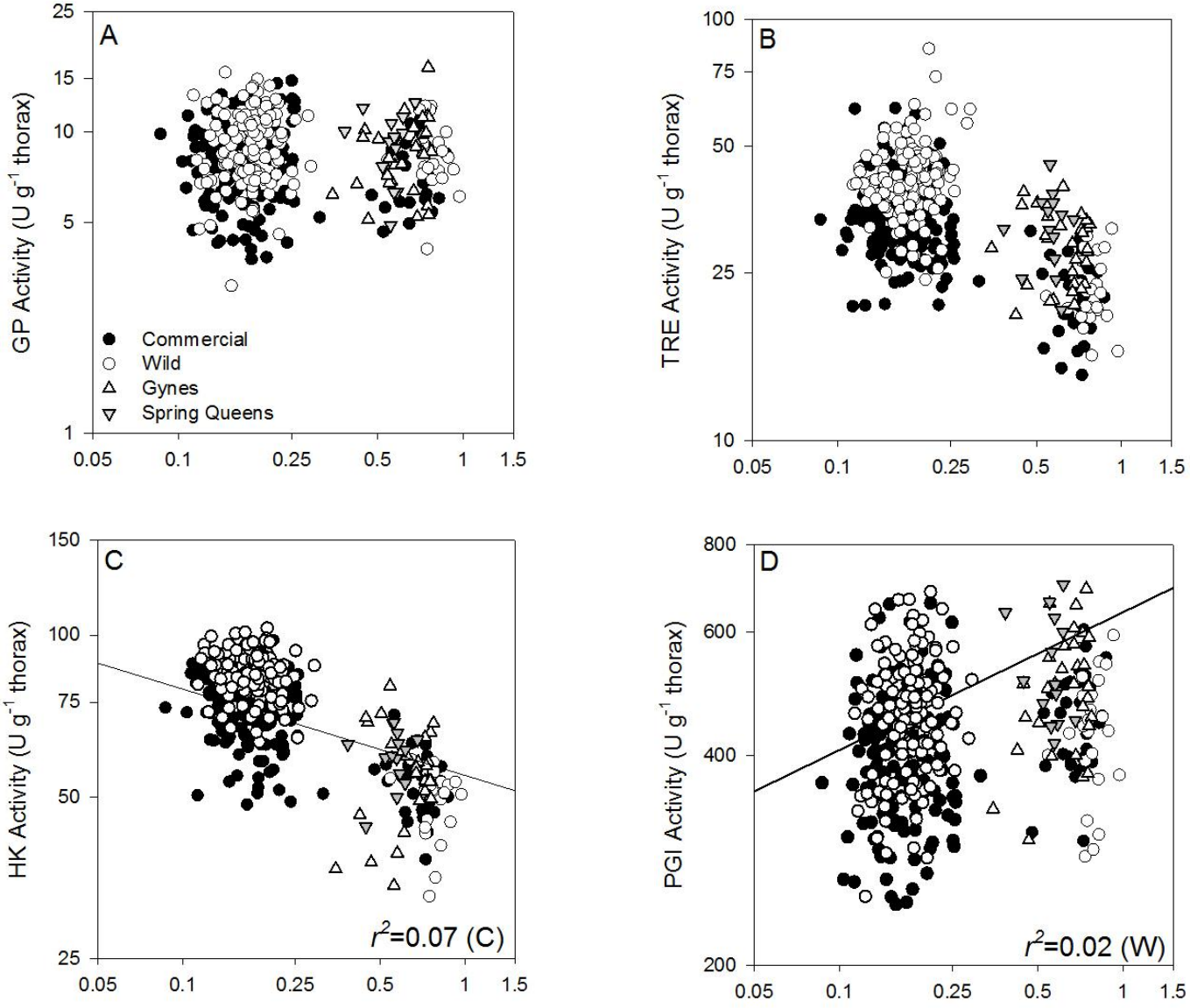

A
GP Activity (U g-1 thorax)
25
15
10
5
1
0.05
0.1
0.25
0.5
1
1.5
Commercial
Wild
Gynes
Spring Queens
B
TRE Activity (U g-1 thorax)
100
75
50
25
10
C
HK Activity (U g-1 thorax)
150
100
75
50
25
r2=0.07 (C)
D
PGI Activity (U g-1 thorax)
800
600
400
200
r2=0.02 (W)
Body Mass (g)

Figure 5.3 Relationship between body mass (g) and (A) glycogen phosphorylase (GP), (B) trehalase (TRE), (C) hexokinase (HK) and (D) phosphoglucoisomerase (PGI) activity values in commercial (closed circles) and wild (open circles) *B. impatiens* workers (n=306) and gravid queens (n=52), gynes (triangles) (G) (n=30) and flying queens collected in the spring (upside down triangles) (SQ) (n=14). Regression data presented for workers only.

5.1.4 Comparison of mean phenotypic values of enzyme activities between groups

We compared workers with gynes in the laboratory (G), young queens collected in the spring (SQ) and gravid queens with established colonies (see Figure 5.4). Using ANCOVAs with body mass as a covariate, analyses revealed that for all enzymes but PGI, there was a significant difference in enzyme activity between groups. Post-hoc comparisons showed that queens had significantly lower activities than workers after taking into account body mass. For GP, workers had higher activities than gravid queens ($p=0.022$). Young queens (G and SQ) have an intermediate value and are not statistically different from workers or gravid queens. Lower TRE activities were also observed in gravid queens when compared to the other groups ($p<0.01$). All queens (young and old) had lower HK activities than workers ($p<0.01$). As for PGI, the younger queens had higher activities than the older ones ($p<0.01$).

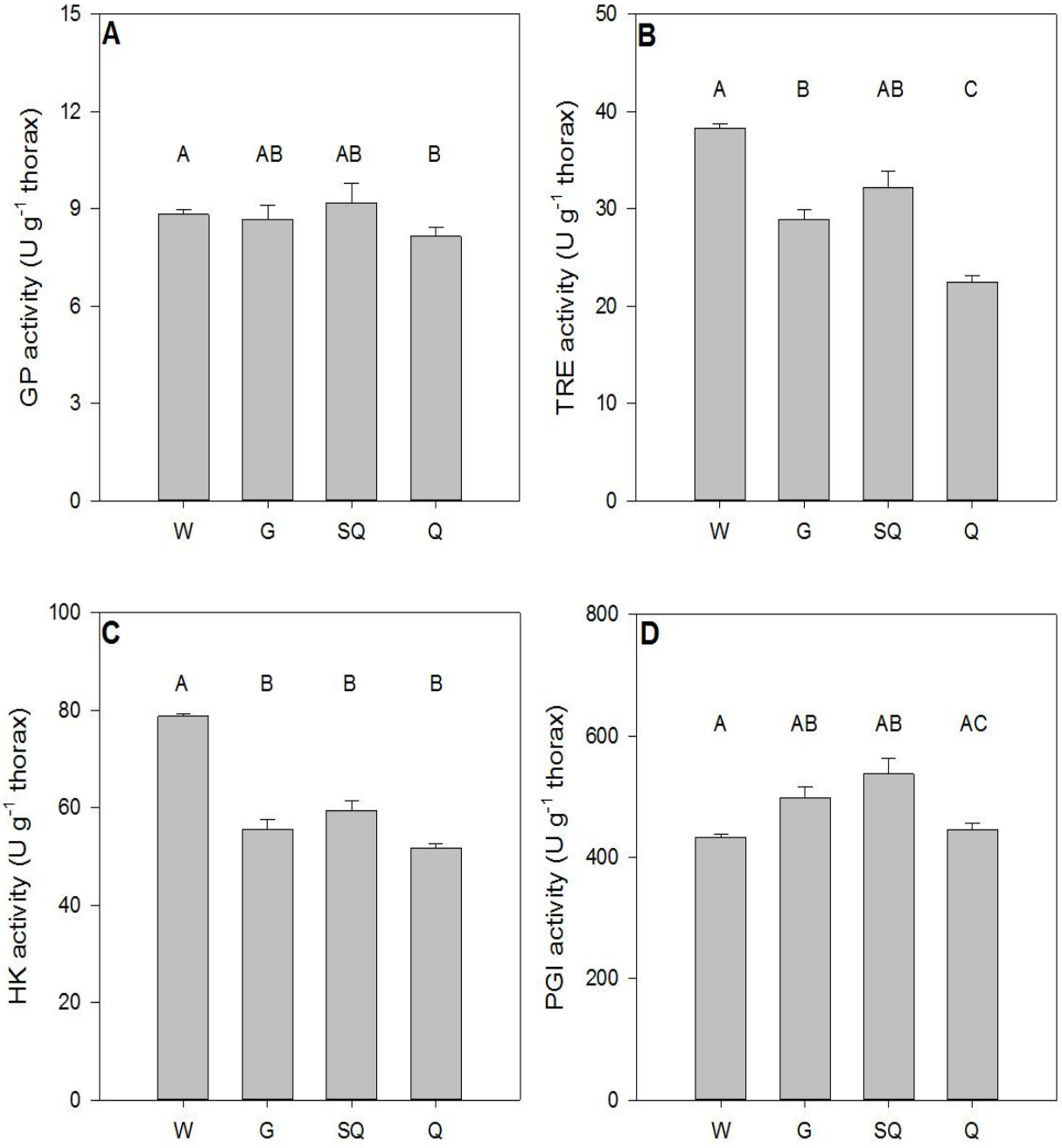

A
GP activity (U g-1 thorax)
15
12
9
6
3
0
A
AB
AB
B
W
G
SQ
Q
B
TRE activity (U g-1 thorax)
50
40
30
20
10
0
A
B
AB
C
W
G
SQ
Q
C
HK activity (U g-1 thorax)
100
80
60
40
20
0
A
B
B
B
W
G
SQ
Q
D
PGI activity (U g-1 thorax)
800
600
400
200
0
A
AB
AB
AC
W
G
SQ
Q

Figure 5.4 Comparison of (A) glycogen phosphorylase, (B) trehalase, (C) hexokinase and (D) phosphoglucoisomerase activities in workers (W) (n=306), gynes (G) (n=30), flying queens collected in the Spring (SQ) (n=14) and gravid queens (Q) (n=52). Letters correspond to the Tukey post-hoc analysis results. Different letters mean that the groups were statistically different ($p<0.05$).

5.2 Repeatability and phenotypic variation in queens

We monitored the phenotypic variation of queens at four time periods from the time of capture in the field to several weeks after the colony was established. Figure 5.5 shows how body mass in queens increases from the first measurement, before the establishment of the colony, compared with all subsequent measurements. Queens gained an average of 40% of their body mass from their capture in the field to the last measurement taken (at around 60-80 workers). Both wing beat frequency and flight metabolic rate showed a higher value in measurements taken one week after emergence of 1st worker (Figure 5.5).

In order to evaluate the repeatability of the different traits, we calculated the intraclass correlation coefficient (τ). In the 16 queens measured at the four time periods, we found that repeatability of FlightMR and WBF approached significance (Table 5.3).

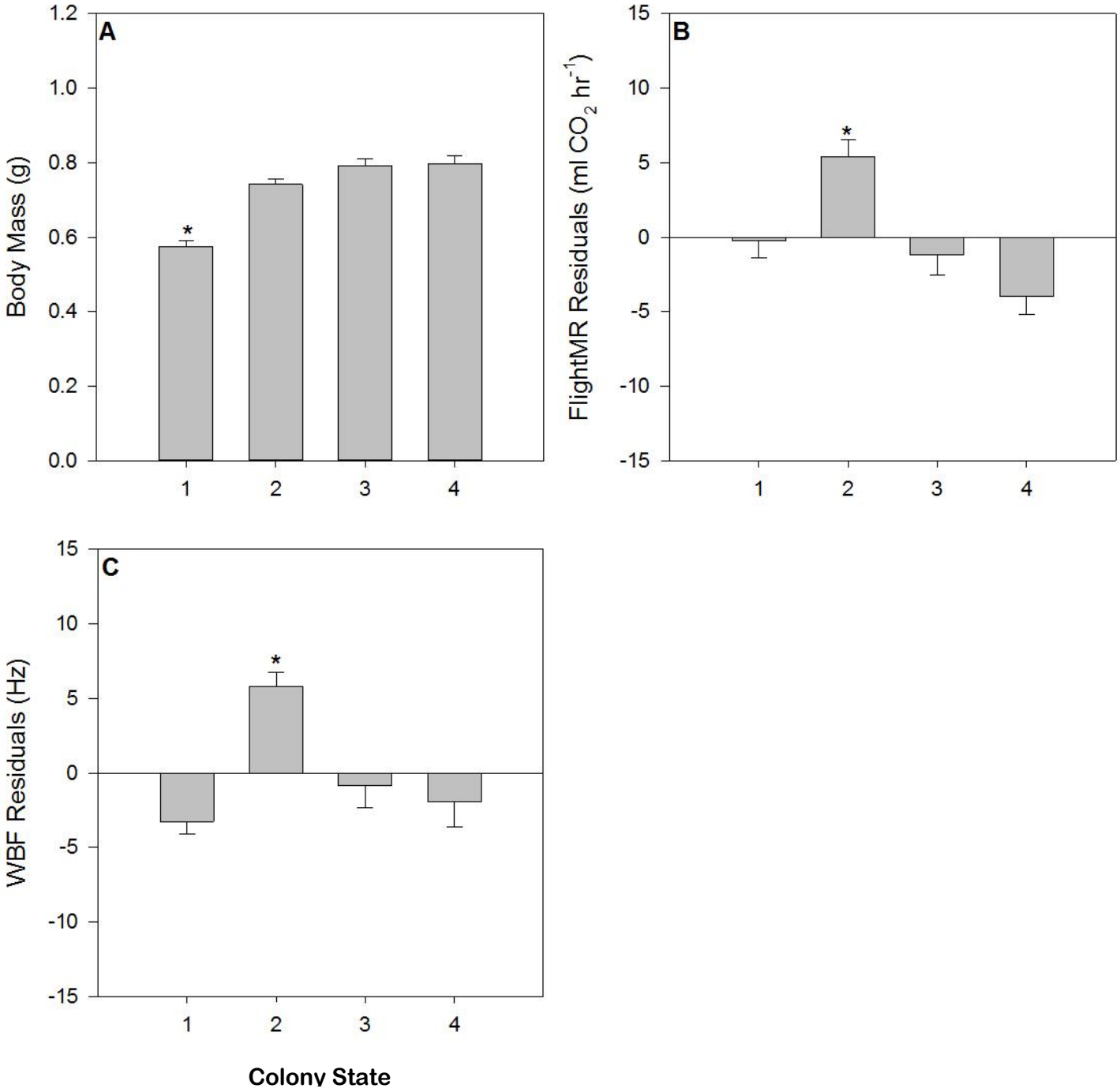

A
Body Mass (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
1
2
3
4
B
FlightMR Residuals (ml CO_2 hr^{-1})
15
10
5
0
-5
-10
-15
*
1
2
3
4
C
WBF Residuals (Hz)
15
10
5
0
-5
-10
-15
*
1
2
3
4
Colony State

Figure 5.5 Mean values (± SE) of (A) body mass (g), (A) flight metabolic rate (FlightMR) (ml CO_2 hr^{-1}), and (C) 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. Asterisk indicates a significant difference from other groups ($p<0.05$).

Table 5.3 Repeatability of Body Mass, FlightMR (ml CO_2 hr^{-1}) and WBF (Hz) as intraclass correlation coefficient (τ) obtained from variance components in a one-way ANOVA (for body mass) and in an ANCOVA using body mass as a covariate in 16 *B. impatiens* queens. Sum of squares (SS) is presented.

Trait	Variance components				
	Between queens SS	Within queens SS		P	τ
Body Mass	0.13	0.13	$F_{15,48}$=0.993	0.477	0.0
FlightMR	52.53	29.16	$F_{15,47}$=1.801	0.063	0.17
WBF	56.15	30.14	$F_{15,45}$=1.863	0.055	0.18

5.3 Heritability Estimates

5.3.1 Narrow-sense heritability estimates

Narrow-sense heritability estimates (h^2) obtained from parent-offspring regressions are presented in Table 5.4. For all traits measured, h^2 was not significantly different from zero (*P*-values from 0.080 to 0.907). Although parent-offspring regressions yielded non-significant results, interfamily differences for most traits were considerable. The range in family values is presented in Table 5.5.

5.3.2 Broad-sense heritability estimates

Broad-sense heritability estimates (H^2) obtained from the full-sib analyses are given in Table 5.5. Full-sib heritabilities of body mass were significantly different from zero and similar in both populations. Heritability of wing surface area accounting for body mass variation, was also significant in both populations. Heritability values of whole-animal flight performance traits (FlightMR and WBF) were both significantly different from 0. H^2 of FlightMR was relatively low, with values of 0.15 ± 0.07 ($p<0.001$) (C) and 0.18 ± 0.08 ($p<0.001$) (W), while WBF values were slightly higher (H^2= 0.26 ± 0.09, $p<0.001$ and H^2= 0.24 ± 0.09, $p<0.001$). H^2 of RMR was one of the highest we obtained, with a value of 0.38 ± 0.14 for commercial colonies ($p<0.001$) (values unavailable for wild colonies). At the cellular metabolic level, glycogen phosphorylase (GP) in commercial colonies and hexokinase (HK) in wild colonies had heritability estimates that were different from zero

(H^2=0.19 ± 0.13, p=0.04 and H^2=0.27 ± 0.15, p=0.012, respectively). Finally, post-hoc analyses revealed that of all of possible family comparisons, 30% of the families were significantly different for body mass, 10% for WSA, 22-24% for FlightMR, 26% for WBF, 12% for GP and 12% for HK.

Table 5.4 Narrow-sense heritability estimates (h^2) with standard error (SE) obtained from a parent- offspring regression performed on body mass, wing section area (WSA), flight metabolic rate (FlightMR), resting metabolic rate (RMR), wing beat frequency (WBF) and enzyme (GP, TRE, HK and PGI) activities. Values are presented for commercial and wild colonies of *B. impatiens*. Sample size (N) is the number of families (colonies) used for each regression.

	Commercial						*Wild*					
Trait	*F*	*P*	r^2	Slope (SE)	h^2 (SE)	N	*F*	*P*	r^2	Slope (SE)	h^2 (SE)	N
Body mass	0.483	0.493	0.019	-0.134 (0.193)	0.268 (0.386)	27	1.109	0.308	0.065	0.259 (0.246)	0.518 (0.492)	18
WSA	0.152	0.700	0.078	-0.075 (0.191)	0.150 (0.383)	27	0.001	0.971	0.009	-0.009 (0.257)	-0.018 (0.438)	17
FlightMR	2.169	0.153	0.08	0.283 (0.192)	0.566 (0.384)	27	2.030	0.135	0.113	-0.335 (0.235)	-0.670 (0.470)	18
RMR	1.236	0.278	0.041	0.231 (0.207)	0.462 (0.414)	24	-[a]	-	-	-	-	-
WBF	0.095	0.760	0.004	0.062 (0.200)	0.124 (0.400)	27	0.025	0.877	0.002	-0.039 (0.250)	-0.078 (0.500)	18
GP	0.233	0.634	0.009	0.096 (0.199)	0.192 (0.398)	27	0.642	0.435	0.039	-0.196 (0.245)	-0.392 (0.490)	18
TR	3.319	0.080	0.117	0.342 (0.188)	0.684 (0.376)	27	0.366	0.554	0.022	0.149 (0.247)	0.298 (0.294)	18
HK	0.014	0.907	0.001	0.023 (0.200)	0.046 (0.400)	27	0.193	0.666	0.012	0.109 (0.248)	0.218 (0.496)	18
PGI	0.060	0.808	0.002	0.048 (0.195)	0.096 (0.390)	27	0.298	0.593	0.018	0.135 (0.248)	0.270 (0.496)	18

[a] -: data unavailable for wild colonies.

Table 5.5 Broad-sense heritability estimates (H^2) with standard error (SE) from a full-sib analysis (one-way ANOVA) accounting for body mass variation. H^2 estimates of body mass, wing section area (WSA), flight metabolic rate (FlightMR), resting metabolic rate (RMR), wing beat frequency (WBF) and enzyme (GP, TRE, HK and PGI) activities are presented for commercial and wild colonies of *B. impatiens*. Range of phenotypic values for families presented for each trait. The number of families (N), the total number of individuals (*n*) and the *p*-value (*P*) are also shown. Values significant at $\alpha = 0.05$ are marked in bold.

	Commercial					*Wild*				
Trait	Family Range[a]	N	*n*	P	H^2(SE)	Family Range[a]	N	*n*	P	H^2(SE)
Body Mass (g)	0.143 - 0.212	28	470	0.000	**0.39 (0.11)**	0.150 - 0.228	26	352	0.000	**0.36 (0.11)**
WSA (mm^2)	4.97 - 7.06	28	268	0.000	**0.27 (0.13)**	4.90 - 6.88	21	194	0.013	**0.19 (0.11)**
FlightMR (ml CO_2 hr^{-1})	10.82 - 15.85	28	470	0.000	**0.15 (0.07)**	11.15 - 16.47	23	352	0.000	**0.18 (0.08)**
RMR (ml CO_2 hr^{-1})	0.23 – 0.39	25	195	0.000	**0.38 (0.14)**	-[b]	-	-	-	**-**
WBF (Hz)	182- 206	28	469	0.000	**0.26 (0.09)**	281 - 209	26	351	0.000	**0.24 (0.09)**
GP (U g^{-1})	4.77 - 12.08	28	162	0.040	**0.19 (0.13)**	7.64 - 11.39	24	144	0.716	-0.07 (0.09)
TRE (U g^{-1})	24.86 - 49.12	28	162	0.203	0.08 (0.12)	37.09 - 49.64	24	144	0.453	0.01 (0.11)
HK (U g^{-1})	58.00 - 84.96	28	162	0.181	0.09 (0.12)	75.47 - 90.70	24	144	0.012	**0.27 (0.15)**
PGI (U g^{-1})	293.82 - 544.52	28	161	0.057	0.26 (0.14)	382.62 - 509.46	24	144	0.497	-0.01 (0.10)

[a] The lowest and highest mean family values are presented.
[b] Data unavailable for wild colonies.

6. DISCUSSION

6.1 Phenotypic values

B. impatiens individuals vary considerably in body size and variations in physiological phenotypes are often associated with body mass. In bumblebee workers, wing surface area seems to scale isometrically with body mass (Buchwald and Dudley, 2010; Skandalis and Darveau, 2012). This relationship was also supported in our study. We obtained a scaling exponent of a wing measure that was not significantly different from 2/3, which is what is expected in a surface area trait. Note that the data presented in Figure 5.1A are surface areas of a wing section used to estimate and compare areas even in the presence of wing wear in our queens. Data available for workers show that the total surface area and this section surface area are highly correlated with an almost isometric relationship (0.94). The section area is therefore a slight underestimate of whole wing surface area, which should bring the slope value closer to isometry.

The effect of body mass on metabolic rate, within and across species, is a subject of debate in biology. Numerous studies have looked at scaling of metabolic rate, but no clear law seems to explain the scaling exponent (see review by Glazier, 2005). In insects, it was recently claimed that metabolic rate should scale according to a ¾ power as predicted by an oxygen supply limitation model (Riveros and Enquist, 2011). However, other analyses in many taxa did not support this claim and showed differential scaling at rest and during flight (Niven and Scharlemann, 2005). Within a group of closely related bee species, Darveau et al.

(2005a) found that mass-specific hovering flight metabolic rate followed an allometric relationship (with an exponent of -0.31) with body mass. It was also shown that wing beat frequency scaled with an exponent identical to hovering flight metabolic rate, suggesting a functional association between flight kinematics and metabolic energy supply. Within species, previous work did not detect a scaling exponent of whole-animal metabolic rate different from 1 in *B. impatiens* workers (Skandalis and Darveau, 2012). In the present study using a larger sample size, we found that hovering flight metabolic rate scales allometrically in workers, with an exponent of 0.83 (or -0.17 when expressed on a mass-specific basis). It is noteworthy that we obtain almost the exact same relationship with wing stroke frequency, which follows a negative exponent of -0.16 for commercial colonies and -0.17 for wild ones. It appears that a decrease in wing beat frequency with body size, is paralleled by a decrease in metabolic rate, similar to the observation across species made by Darveau et al. (2005a). This parallel scaling within species is further supporting the strong association between wing beat frequency and metabolic rate in bees. Furthermore, Casey et al (1985) and Darveau et al (2005a) showed that differences in mass-specific metabolic rate of bee genera or species having similar body sizes was related largely to differences in wing stroke frequency and this relationship was also supported at the intraspecific level in bumblebees (Skandalis and Darveau, 2012). This association between size-independent metabolic rate and wingbeat frequency holds true in our study (Figure 5.2 B), in addition to the inverse relationship between residual wingbeat frequency and wing surface area (or WSA used as proxy for total wing surface area; Figure 5.2A). Therefore, bees with larger wings for their body size will have lower wing beat frequencies than their counterparts, which in turn explain their lower flight metabolic rate. In sum, flight metabolic rate variation in *B. impatiens* can be explained

by variation in wing size influencing wing kinematics, ultimately explaining the scaling of flight metabolic rate.

The relationship between energy metabolism and body mass was not only observed while bees were flying, but also at rest (see Figure 5.1). In fact, resting metabolic rate scaled allometrically with an exponent almost identical to that of metabolic rate during flight (0.85 compared to 0.83; see Table 5.1). Scaling exponents obtained in this study were similar to what was reported in some species of beetles (0.84 at 20ºC in Rogowitz and Chappell, 2000), above values reported in moths (0.76 in Bartholomew and Casey, 1978) or for insects as a group (Niven and Scharlemann, 2005), and within the range reported for various insect families (Riveros and Enquist, 2011). Other studies, such as Terblanche and Anderson (2010), report isometric relationships between RMR and body mass in other pollinator species (the hawkmoth *Macroglossum trochilus* and the fly *Moegistorhynchus longirostrus*), but intraspecific metabolic rate scaling typically shows broader variation ranging from isometric to allometric (Glazier, 2005). To date, there is no consensus regarding the relationship between resting and exercise metabolic rates. However, some studies performed in birds and mammals suggest a functional relationship between the two. For instance, Rezende et al (2004) found a positive correlation between basal and maximal metabolic rates (after statistically accounting for body size) across species of rodents. Sadowska et al (2004) tested the relationship between both traits at the genetic level in bank voles. They found a positive additive genetic correlation between basal metabolic rate and swim-induced aerobic capacity. The similarity in scaling exponents found in our study suggest a functional relationship between resting and exercise metabolic rate, in which animals with higher energy metabolism at rest would also have higher metabolic rates during flight. Further work

is required in order to draw stronger conclusions as our study design was conceived to obtain mid-offspring values and not individual data. We therefore used the same group of bees for RMR and FlightMR measurements, but did not tag them individually, so that linking the two values was not possible. It could be interesting to assess the correlation between the two and the individual variation in aerobic scope in this species.

The effect of mass on metabolic phenotypes of animals is not limited only to whole-animal properties, it is also reflected at the cellular level (Emmett and Hochachka, 1981; Somero and Childress, 1990; Davies and Moyes, 2007). In *B. impatiens*, hexokinase activity decreases with body mass (Skandalis and Darveau, 2012), an observation that is supported by our findings. Hexokinase activity is of particular interest in the context of flight energetics as it is correlated with flight metabolic rate both within (Skandalis and Darveau, 2012; Darveau et al., in press) and across species of bees (Darveau et al., 2005). Darveau et al. (in press), also report associations between flight metabolic rate and other glycolytic and mitochondrial enzyme activities. Although we did not detect these relationships in our data set, hexokinase decreased with body mass and differences between populations were observed (see Figure 5.3 and Table 5.1). Of the other enzymes, only phosphoglucoisomerase scaled positively with body mass. However, this enzyme activity was very variable and the proportion of variance explained by the relationship was very small. Close associations between wing stroke frequency and mass-specific metabolic rate and similar scaling of hexokinase activity suggests that there is an association between flight performance and the cellular metabolic phenotype at this particular step of glycolysis.

The comparison of commercially acquired bee colonies and colonies reared from wild caught queens yielded interesting results. Scaling exponents of wing surface area, flight metabolic rate and wing beat frequency was not significantly different in both groups. Intercept differences were detected in some of the traits of interest (see Table 5.1). For instance, significant differences were observable in body mass, wing surface area and wing beat frequencies. These differences were very small (less than 3%) and possibly due to the large sample size used in the comparison. When comparing both populations' muscle metabolic phenotype, wild colonies had higher activities for all enzyme studied (Figure 5.3). The most striking differences were observed in hexokinase and trehalase activities, which were 15% and 20% higher in wild colonies. In the Colias butterfly, differences in flight performance (i.e. dispersal) between populations have been associated with one gene coding for the glycolytic enzyme phosphoglucoisomerase (Watt et al., 2003). Differences between populations support the idea that the metabolic phenotype is partially genetically determined. Another possible explanation would be that there is inbreeding depression in commercial colonies. Bumblebees are susceptible to inbreeding depression due to population declines and loss of flower rich meadows to agriculture. In honeybees, flight muscle enzyme activities decrease as a result of inbreeding (Moritz, 1983). Loss of genetic diversity has been shown to reduce fitness in bumblebee colonies (Whitehorn et al., 2009). In crickets, inbreeding affects energy allocation (Ketola and Kotiaho, 2009). If inbreeding has an effect on the flight phenotype by decreasing flight performance of workers, this could have long term repercussions. Although we did not assess the level of inbreeding in our sample, we would suppose that it would be higher in commercial colonies. This could explain why commercial colonies had consistently lower enzyme activities than their wild counterparts. Assessing the amount of inbreeding in commercial bumblebee populations would be an interesting follow-

up to this study, especially considering potential relationships between muscle phenotype and flight performance. Bumblebees are commonly sold for pollination purposes in greenhouses, this information could therefore be important for commercial breeders.

6.2 Repeatability and phenotypic variation in queens

Although the flight phenotype of bumblebee workers has been fairly well studied, very little is known about flight parameters in queens. In *B. impatiens*, workers and queens differ in size and physiology, but not in general morphology (Heinrich, 2004). Queens are formed at a later stage of the colony, and their larval growth rate does not differ from workers. Queens have longer larval stages and therefore a longer feeding period, resulting in larger adult sizes (Cnaani et al., 1997). However, it is still not clear whether queens are in fact "larger workers". We were able to take measurements in commercial and wild queens and at different stages of their lives. Morphologically, queens are much larger than workers (about 4.5 times). Our results show that the queens' wing surface area scaled positively with an exponent of 0.20 (Table 5.2). Although this relationship is not as clear as what was observed in workers, the sample size was much smaller (44 compared to 462) and the range in size was also reduced. To avoid bias introduced by analyzing separate clusters of data, we show regressions for workers only (see Figure 5.1). Queens seem to follow the relationship which was predicted in workers for wing size.

One of the major observations when taking flight measurements in queens was the apparent decrease in flight ability over time. Queens collected in the spring flew very well and their hovering flight metabolic rate fell on the path predicted by scaling in workers

(Figure 5.1 B). However, the gravid queens with established colony fell under the regression line, with lower flight metabolic rates than what was expected for their size based on the relationship in workers.

Wild queens gained an average of 40% of their body mass over the time of the study, and body mass was not repeatable (Figure 5.5 and Table 5.3), indicating that larger queens in the spring were not necessarily comparatively larger towards the end of the season. It is also important to note that in order to avoid perturbation in the colony by removing the queen for a long period of time, we performed resting metabolic rate measurements in queens at an even later stage of the colony and queens kept increasing in size resulting in an average weight of over 1 gram. We suggest that this weight gain is probably due to the growth and maturation of the ovaries. Vogt et al. (1998), reported that queens provided with pollen and nectar *ad libitum* tripled the mass of their ovaries in just 8 days. In insects, fecundity is generally highly dependent on body size (Honěk, 1993). Higher investments in gametes results in higher chances of having numerous offspring and therefore, higher fitness. The consequence of increased gamete production is higher weight loads and reduction in mobility which could result in impaired predator avoidance for example (Magnhagen, 1991). The higher weight loads associated with reproduction have been shown to reduce flight performance in insects (Almbro and Kullberg, 2012).

Although measurements of wing surface area were only performed after dissection, we qualitatively observed wing wear over time in queens. Over the course of their lives, flying insects accumulate wing wear and have no mechanisms to repair wing damage (Hayes and Wall, 1999; Higginson and Barnard, 2004). Foster and Cartar (2011) showed that

bumblebees with a higher frequency of wing collisions had an increased loss of wing area, which became more severe over time. Wing wear has been shown to increase wing beat frequency (Hedenström et al, 2001) and affects other aspects of flight including speed and performance (Fischer and Kutsch, 2002; Haas and Cartar, 2008; Combes et al., 2010). Previous work suggests that some of the major determinants of flight performance are wing loading and flight muscle ratio (thorax mass/body mass) (Marden, 1987; Ellington, 1991; Berwaerts and Van Dyck, 2004). Studies performed on bees at the intraspecific and interspecific level also report an association between flight cost (measured by metabolic rate) and wing loading (Darveau et al., 2005; Skandalis and Darveau, 2012). Darveau et al. (in press) found significant differences in flight phenotypes of drones and workers of the same colony related to lower wing loading resulting in lower metabolic rates during flight. The increased body mass of queens in combination with wing wear results in wing loading that might be too high in order to sustain flight. In figure 5.5, we can see that the second measurement of flight metabolic rate and wing beat frequency in queens was higher than all other measurements. It is possible that the increase in body mass led to a compensation in wing beat frequency, resulting in higher metabolic rates for the second measurement.

Repeatability of flight metabolic rate and wing beat frequency has been shown to be high in *B. impatiens* workers (Darveau et al., in press). As seen in table 5.3, repeatability of these traits approached significance for flight metabolic rate and wing beat frequency. In queens however, body mass was not repeatable which indicates that it is a trait with large phenotypic plasticity. *B. impatiens* queens are required to fly at the beginning of the season, when the number of workers in the colony is insufficient to fulfill colony duties. As the season progresses, the queens will lay more eggs at a time and more frequently, increasing

energy allocation to reproduction and will rarely exit the colony (Heinrich, 2004). We suggest that the apparent decrease in flight ability is a trade-off between mobility and fecundity. Since queens have no need to exit the colony, they produce more eggs and become larger, impeding their ability to fly.

When comparing queens at different life stages and workers, differences are noticeable at the cellular metabolic level. In gynes, young queens and gravid queens, hexokinase is significantly lower than in workers even after taking into account size differences (using ANCOVAs) (Figure 5.3 and 5.4). Glycogen phosphorylase and trehalase activity are also inferior in gravid queens than in workers and seems to decrease over life stages (from gynes to gravid queens). Enzyme activities have been correlated with flight metabolic rate and wing loading in this species. Drones have lower wing loading, metabolic rates and enzyme activities. The flight muscle phenotype seems to vary with other flight traits and between groups of bees which is consistent with previous findings in bees (Darveau et al., 2005; Skandalis and Darveau, 2012; Darveau et al., in press).

Judice et al. (2004), compared gene expression in queens and workers of a species of stingless bees. They found differential expression of genes coding for proteins that are essential for the flight muscle structure of bees. They suggest that an overexpression of these genes relates to the fact that workers heavily invest in flight muscle structural proteins in order to support long foraging flights in the course of their lives. In contrast, queens of this species wouldn't require the same investment as they seldom exit their colony after its establishment. Previous studies have suggested a possible trade-off between flight and fecundity that could be due to a pleitropic relationship between genes for flight capacity and

genes for fecundity (Schumacher et al., 1997). It is possible that this is why we observe differences between workers and queens in our study.

6.3 Heritability

The main goal of this study was to investigate heritability of flight performance and its associated traits in bees. Various studies have evaluated heritability of morphological traits related to flight such as wing surface area, wing shape and wing length in insects, including fruit flies (H^2=0.33, Curtsinger and Laurie-Ahlberg, 1981; h^2= 0.71, Moraes and Sene, 2004), crickets (H^2=0.25, Bégin and Roff, 2002) and moths (H^2=0.70, Keena et al., 2007). Heritability estimates obtained from our parent-offspring regressions were not significantly different from zero for all traits (see Table 5.4). However, when performing full-sib analyses, we found significant broad-sense heritability estimates for body mass (H^2=0.39 and 0.36) and wing morphology (H^2=0.27 and 0.19) in both populations (see Table 5.5). Heritability estimates of body mass in this context could be inflated by common environment. However, it is important to note that body mass was included in the analysis of variance for wing morphology. This means that the ratio of wing surface to body size is genetically determined and could be subject to natural selection.

The studies of heritability of flight performance in insects generally focus on flight duration and flight distance and estimates vary greatly between studies. Heritability of flight distance has been estimated in different species of moths (h^2=0.4, Parker and Gatehouse, 1985; h^2=0.27, Han and Gatehouse, 1993; h^2=0.38, Schumacher et al., 1997). Flight duration is heritable in beetles (h^2=0.125-0.531, Tanaka, 2009), fruit flies (H^2=0.21-0.24, Gu and

Barker, 1995) and moths (h^2=0.56, Gu and Danthanarayana, 1992). Heritability estimates of metabolic rate during flight in insects are not available, but in other animals during locomotion maximal oxygen consumption rate appears to be heritable in some species of reptiles (H^2=0.88, Garland and Bennett, 1990) and mammals (h^2=0.64, Dohm et al., 2001), although some studies report non significant estimates (Nespolo et al., 2003). To our knowledge, our study is the first to report heritability values of flight metabolic rate and its correlated traits in insects. Our results show significant heritability estimates for flight metabolic rate using full-sib analyses (H^2= 0.15 and 0.18 for commercial and wild colonies respectively). Wing beat frequency was also heritable and with similar values in both populations (H^2=0.26 in commercial colonies and 0.24 in wild colonies). Components of flight morphology and flight performance are therefore in part genetically predetermined in *B. impatiens.* This would mean that workers of a same colony could be better flyers because of their genetic makeup.

We were not only interested in looking at energy metabolism during flight, but also at rest. Resting metabolic rate has been mostly studied in endothermic vertebrates such as birds and mammals (Dohm et al., 2001; Nilsson et al., 2009; Tielman et al., 2009). Reported heritabilities for resting metabolic rates are typically low (Dohm et al, 2001; Nespolo et al., 2003; Rønning et al., 2007; Ketola and Kotiaho, 2009), which is expected of fitness related traits. These traits are generally under strong selection and therefore exhibit reduced genetic variability and lower heritabilities. However, some studies have found significant heritability values, for instance in birds (h^2=0.59, Nilsson et al., 2009; h^2=0.36, Tielman et al., 2009) and mammals (h^2=0.4, Sadowska et al., 2005, h^2=0.32-0.61, Zub et al., 2012). In insects, low but significant broad-sense heritabilities of resting metabolic rate were reported (H^2=0.052,

Nespolo et al., 2007) as well as non significant ones (Piiroinen et al., 2011). We are the first to report significant heritability values for resting metabolic rate in bees (0.38), which is relatively high in comparison with other traits of interest in our study. Resting metabolic rate is a common measure of the cost of "maintenance" in animals. As mentioned previously, our data shows a possible functional relationship between resting metabolic rate and hovering flight metabolic rate in bees. This would mean that individuals exhibiting higher metabolic rates for activity would also exhibit higher metabolic rates at rest. In our study, both measurements were heritable and suggest that determination of metabolic rate is in part genetic, despite high phenotypic variation.

Muscle metabolic phenotypes have been studied in the context of heritability of locomotor performance. Muscle metabolic enzyme activity has been shown to be heritable in some groups (fish: Garenc et al., 1998; mammals: Larzul et al., 1997; insects, Pecsenye et al., 2004), but there is still little information available on heritabilities of glycolytic enzymes in the context of activity metabolism. We obtained broad-sense heritabilities estimates that varied between populations. Heritability of glycogen phosphorylase was significant in commercial colonies, whereas hexokinase was significantly heritable in wild colonies. The repeatability of these traits in bees has not been assessed, as the relatively small size of the flight muscle would make it impossible to perform assays using biopsies. However, these traits exhibit large interindividual variation (Skandalis and Darveau, 2012; Darveau et al., in press) and high phenotypic plasticity could make it difficult to detect significant heritability. Larger sample sizes could be used to assess this in future work.

It is possible that full-sib analysis leads to an overestimation of heritability due to maternal effects or common environment for siblings (Falconer and Mackay, 1996). Previous studies have reported higher heritabilities when using the full-sib method, compared to the parent-offspring regressions (Blanckenhorn, 2002; Müller et al., 2003). However, other studies have reported that there is no bias introduced by the method of heritability estimation (Mousseau and Roff, 1987; Schumacher et al., 1997). The similarities between heritability estimates of both groups indicate that our estimates were not overestimates. Indeed, we would have expected environmental variance due to maternal effects to be higher in wild colonies, as they were generated from wild-caught queens.

One of the possible explanations of the lack of narrow-sense heritability would be that the proportion of environmental variance (V_E) was high. However, this is very unlikely, as Darveau et al. (in press) reported high values of repeatability for body mass, flight metabolic rate and wing beat frequencies in bumblebee workers. A more likely explanation resides in the fact that we suspect that the traits of interest in queens are variable over time, as mentioned earlier. If queens express a different phenotype than their workers, then an accurate estimation of heritability through parent-offspring regression would be impossible to obtain. In order to obtain valid heritability values, the same trait must be measured in the parent and subsequently in the offspring. Our results suggest that this was not the case, and could explain the lack of significance of our analyses.

7. CONCLUSION

As a first step in this study, we wanted to characterize variation in flight metabolic rate and its associated traits. Mass-specific metabolic rate, wing beat frequency and hexokinase had similar scaling exponents, which is consistent with what was observed across species of bees and suggests possible correlated evolution of these traits (Darveau et al., 2005b). Flight metabolic rate and resting metabolic rate scaled allometrically with the same relationship, indicating that there might be a functional relationship between the two traits. We observed interesting differences between wild and commercial populations, in particular at the cellular metabolic level. Wild bees have higher enzyme activities than commercial ones indicating possible genetic differences between populations. The differences observed in flight muscle metabolic phenotype may indicate possible inbreeding depression in commercial colonies similar to what has been observed in commercial colonies of honeybees (Moritz, 1983).

We found significant broad-sense heritability estimates for body mass, relative wing surface area, metabolic rate at rest and during flight and wing beat frequency. We conclude that these traits have some genetic basis, although we are aware that broad-sense heritability estimates could be inflated by factors other than additive genetic variance. The divergence in estimates between narrow-sense and broad-sense heritabilities could indicate that additive effects are not the major source of genetic variation in the observed traits. However, we suggest that bumblebee queens might express a different flight phenotype than their workers,

due to their reproductive demands, making the estimation of narrow-sense heritability using parent-offspring regression difficult to obtain. Further work should be done in order to assess narrow-sense heritability of these traits in bees. Finally, this is the first study to investigate flight performance in queens at different levels of organization. We provide useful information that can be used as a first step towards a better understanding of phenotypic differences in flight ability between workers and queens. Bumblebees are very efficient pollinators and are sold commercially for pollination purposes. Understanding the bases of flight performance is therefore particularly relevant. If selection can act on flight performance traits, then it would eventually be possible to select for colonies of 'better flyers'.

8. REFERENCES

Äkesson, M., Bensch, S., Hasselquist, D., Tarka, M. and Hansson, B. (2008) Estimating heritabilities and genetic correlations: comparing the 'animal model' with parent-offspring regression using data from a natural population. *PLOS* **3**: e1739

Almbro, M. and Kullberg, C. (2012). Weight Loading and Reproductive Status Affect the Flight Performance of Pieris napi Butterflies. *Journal of insect behavior*, **25**(5), 441-452

Artacho, P. and Nespolo, R.E. (2009) Intrapopulational variation in the standard metabolism of a terrestrial mollusc: repeatability of the CO_2 production in the land snail *Helix aspersa*. *Physiological and Biochemical zoology* **82(2)**: 181-189

Bartholomew, G.A. and Casey, T.M. (1978) Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *J Exp Biol.* **76**:11-25

Bégin, M. and Roff, D.A. (2002). The common quantitative genetic basis of wing morphology and diapause occurrence in the cricket *Gryllus veletis*. *Heredity* **89**(6): 473-479

Bech, C., Langseth, I., Gabrielsen, G.W. (1999) Repeatability of basal metabolism in breeding female kittiwakes *Rissa tridactyla*. *Proc. R. Soc. Lond.* **266**(1434):2161-2167

Bennet, P. M., and Harvey, P. H. (1987). Active and resting metabolism in birds: allometry, phylogeny and ecology. *Journal of Zoology* **213**(2), 327-344

Berwaerts, K. and Van Dyck, H. (2004) Take-off flight performance under optimal and suboptimal temperature in the butterfly *Pararge aegeria*. *Oecologia* **141**: 536-545

Bienefeld, K. and Pirchner, F. (1990). Heritabilities for several colony traits in the honeybee (*Apis mellifera carnica*). *Apidologie* **21**:175-183

Bishop, C. M. (1999). The maximum oxygen consumption and aerobic scope of birds and mammals: getting to the heart of the matter. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **266**(1435), 2275-2281

Bitner-Mathé, B.C. and Klaczko, L.B. (1999) Heritability, phenotypic and genetic correlations of size and shape of *Drosophila mediopunctata* wings. *Heredity* **83**: 688-696

Blackenhorn, W.U. (2002) The consistency of quantitative genetic estimates in field and laboratory conditions in the yellow dung fly. *Genetica* **114**:171-182

Blumstein, D.T., Lea, A., Olson, L.E. and Martin, J.G.A. (2010) Heritability of anti-predatory traits: vigilance and locomotor performance in marmots. *Journal of Evolutionary Biology*. **23**(5): 879-887

Boecking, O., Bienefeld, K., and Drescher, W. (2000). Heritability of the Varroa-specific hygienic behaviour in honey bees (Hymenoptera: Apidae). *Journal of Animal Breeding and genetics* **117**(6), 417-424

Boelling, D., and Pollott, G. E. (1998). Locomotion, lameness, hoof and leg traits in cattle II.: Genetic relationships and breeding values. *Livestock production science* **54**(3), 205-215

Buchwald, R. and Dudley, R. (2010) Limits to vertical force and power production in bumblebees (Hymenoptera: *Bombus impatiens*). *J Exp Biol* **213**:426-432

Caldwell, R.L. and Hegmann, G.P. (1969) Heritability of flight duration in the milkweed bug *Lygaeus kalmii*. *Nature* **223**: 91-92

Candy, D.J., Becker, A., Wegener, G. (1997) Coordination and integration of metabolism in insect flight. *Comparative Biochemistry and Physiology* **117**(4):497-512

Casey, T.M., May, M.L. and Morgan, K.R. (1985) Flight energetics of euglossine bees in relation to morphology and wing stroke frequency. *J Exp Biol* **116**:271-289

Chappell, M.A., Bachman, G.C. and Odell, J.P. (1995) Repeatability of Maximal Aerobic Performance in Belding's Ground Squirrels, *Spermophilus beldingi*. *Functional Ecology* **9**(3):498-504

Chappell, M. A. and Rogowitz, G. L. (2000). Mass, temperature and metabolic effects on discontinuous gas exchange cycles in eucalyptus-boring beetles (Coleoptera : Cerambycidae). *J. Exp. Biol.* **203**: 3809-3820

Clarke, A., and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, **68**(5), 893-905

Cnaani, J., Schmid-Hempel, R. and Schmidt, J.O. (1997) Colony development, larval development and worker reproduction in Bombus impatiens Cresson. *Insectes Sociaux* **49**(2):164-170

Combes, S. A., Crall, J. D. and Mukherjee, S. (2010) Dynamics of animal movement in an ecological context: dragonfly wing damage reduces flight performance and predation success. *Biol. Lett.***6**, 426-429

Curtsinger, J. and Laurie Ahlberg, C. (1981) Genetic variability of flight metabolism in *Drosophila melanogaster*. I. Characterization of power output during tethered flight. *Genetics* **98:**549-564

Darveau, C.-A., Billardon, F. and Bélanger, K. Intraspecific variation in flight metabolic rate in the bumblebee *Bombus impatiens*: repeatability and functional determinants in workers and drones. *In press, J. Exp. Biol.*

Darveau, C.-A., Hochachka, P.W, Roubik, D.W., Welch, K. and Suarez, R.K. (2005a) Allometric scaling of flight energetics in panamanian orchid bees: A comparative phylogenetic approach. *J. Exp. Biol.* **208**: 3581-3591

Darveau, C.-A., Hochachka, P.W, Roubik, D.W., and Suarez, R.K. (2005b) Allometric scaling of flight energetics in orchid bees: Evolution of flux capacities and flux rates. *J. Exp. Biol.* **208**: 3593-3602

Davies, R. and Moyes, C. D. (2007) Allometric scaling in centrarchid fish: origins of intra- and inter-specific variation in oxidative and glycolytic enzyme levels in muscle. *J. Exp. Biol.* **210**: 3798-3804

DeVries, Z. C., Kells, S. A., and Appel, A. G. (2013). Standard metabolic rate of the common bed bug, Cimex lectularius: Effects of temperature, mass, and life stage. *Journal of insect physiology* **50**(11):1133-1139

Diniz, J., and Pignata, M. I. B. (1994). Quantitative genetics of multivariate morphometric variation in the neotropical stingless bee, *Scaptotrigona postica* (Hymenoptera, meliponinae). *Revista Brasileira de Genetica*, **17**(3), 259-265

Dohm, M.R. (2002). Repeatability estimates do not always set an upper limit to heritability. *Functional Ecology*, **16**(2), 273-280

Dohm, M. R., Hayes, J. P., and Garland Jr, T. (1996). Quantitative genetics of sprint running speed and swimming endurance in laboratory house mice (Mus domesticus). *Evolution* 1688-1701

Dohm, M.R., Hayes, J.P. and Garland, T. Jr. (2001) The quantitative genetics of maximal and basal rates of oxygen consumption in mice. *Genetics* **159**:267-277

Ellington, C.P. (1991) Limitations on animal flight performance. *J Exp Biol.* **160**:71-91

Emmett, B. and Hochachka, P.W. (1981) Scaling of oxidative and glycolytic enzymes in mammals. *Respiration Physiology* **45**: 261-272

Endler, J.A. (1986) Natural selection in the wild. *Princeton University Press*, Princeton, New Jersey.

Falconer, D.S. and Mackay, T.F.C. (1996) Introduction to Quantitative Genetics. 4th edn. Longman, New York

Fischer, H. and Kutsch, W. (2002) Relationships between body mass, motor output and flight variables during free flight of juvenile and mature adult locusts, *Schistocera gregaria*. *J. Exp. Biol.* **203**:2723-2735

Foster, D.J. and Cartar, R.V. (2011) Wing wear affects wing use and choice of floral density in foraging bumblebees. *Behav. Ecol.* **22**:52-59

Fournier, F. and Thomas, D.W. (1999) Thermoregulation and repeatability of oxygen-consumption measurements in winter- acclimatized North American porcupines (Erethizon dorsatum). *Canadian Journal of Zoology* **77**(2): 194-202

Francoy, T.M., Silva, R.A.O., Nunes-Silva, P., Menezes, C., Imperatriz-Fonseca, V.L. (2009) Gender identification of five genera of stingless bees (Apidae, Meliponini) based on wing morphology.*Genetics and Molecular Research* **8**(1): 207-214

Freeman, S. and Herron, J.C. (2007). Evolutionary analysis. *Pearson Prentice Hall.* 834 p.

Friedman, W. A., Garland, T. and Dohm, M. R. (1992) Individual variation in locomotor behavior and maximal oxygen consumption in mice. *Physiol. Behav.* **52**, 97-104

Fyhn, M., Gabrielsen, G. W., Nordoy, E. S. and Moe, B. (2001) Individual variation in field metabolic rate of kittiwakes (*Rissa tridactyla*) during the chick-rearing period. *Physiol. Biochem. Zool.* **74**, 343-355

Garenc, C., Siversides, F.G. and Guderley, H. (1998). Burst swimming and its enzymatic correlates in the threespine stickleback (*Gasterosteus aculeatus*): full-sib heritabilities. *Can J Zool.* **76**:680-688

Garland, T. Jr. (1984) Physiological correlates of locomotory performance in a lizard: an allometric approach. *American Journal of Physiology* **247**(5):R806-815

Garland, T. Jr. (1988) Genetic basis of activity metabolism.I. Inheritance of speed, stamina, and antipredator displays in the garter snake *Thamnophis sirtalis. Evolution* **42**: 335-350

Garland, T. Jr. and Bennett, A. F. (1990) Quantitative genetics of maximal oxygen consumption in a garter snake. *Am. J. Physiol.* **259**, R986-R992

Glazier, D.S. (2005) Beyond the '3/4-power law': variation in the intra-and interspecific scaling of metabolic rate in animals. *Biological Reviews* **80**(4):611-662

Gu, H. and Barker, J.S.F. (1995) Genetic and phenotypic variation for flight ability in the cactophilic *Drosophila* species, *D. aldrichi* and *D. buzzatii. Entomologia Experimentalis et Applicata* **76**: 25-35

Gu, H., and Danthanarayana, W. (1992) Quantitative genetic analyses of dispersal in *Epiphyas postvittana* (Walker). I. Genetic variation in flight capacity.*Heredity* **68**: 53–60

Haas, C. A. and Cartar, R. V. (2008) Robust flight performance of bumble bees with

artificially induced wing wear. *Can. J. Zool*. **86**, 668-675

Han, E. and Gatehouse, G. (1993) Flight capacity: genetic determination and physiological constraints in a migratory moth *Mythimna separata.Pysiol. Entomol.***18**: 183-188

Han, L.Z., Gu, H.N., Zhai, B.P. and Zhang, X.X. (2009) Genetic effects on flight capacity in the beet armyworm, *Spodoptera exigua* (Lep., Noctuidae). *Journal of Applied Entomology* **133**(4):262-271

Hayes, J.P. and Jenkins, S.H. (1997) Individual Variation in Mammals. *Journal of Mammalogy* **78**(2):274-293

Hayes, E.J. and Wall, R. (1999) Age-grading adult insects: a review of techniques. *Physiol. Entomol.* **24**:1-10

Heinrich, B (2004) Bumblebee economics. *Harvard University Press*, Cambridge, Massachusetts

Hedenström, A., Ellington, C.P., and Wolf, T.J. (2001) Wing wear, aerodynamics and flight energetics in bumblebees (*Bombus terrestris*): an experimental study. *Funct. Ecol.* **15**, 417-422

Higgison, A.D. and Barnard, C.J. (2004) Accumulating wing damage affects foraging decisions in honeybees (*Apis mellifera L*) *Ecol. Entomol.* **29**:52-59

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66:**483-492

Horak, P., Saks, L., Ots, I., Kollist, H. (2002) Repeatability of condition indices in captive greenfinches (Carduelis chloris). *Canadian Journal of Zoology* **80**(4):636-643

Irschick, D.J. and Garland, T. Jr. (2001) Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annual Review of Ecology and Systematics* **32**:367-396

Jevtić, G., Anđelković, B., Lugić, Z., Radović, J., and Dinić, B. (2012). Heritability of production characteristics of regional populations of honey bees from Serbia. *Genetika* **44**(1), 47-54

Judice, C., Hartfelder, K. and Pereira, G.A.G. (2004) Caste-specific gene expression in the stingless bee *Melipona quadrifasciata* – Are there common patterns in highly eusocial bees? *Insect. Soc.* **51**:352–358

Keena, M.A., Grinberg, P.S. and Wallner, W.E. (2007) Inheritance of female flight in *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environmental Entomology* **36**(2):484-494

Ketola, T. and Kotiaho, J.S. (2009) Inbreeding, energy use and condition. *J Evol Biol.* **22**:770-781

Knott, S.A., Sibly, R.M., Smith, R.H. and Moller, A.P. (1995) Maximum likelihood estimation of genetic parameters in life-history studies using the 'animal model'. *Functional Ecology* **9**: 122-126

Koch, L. G., Meredith, T. A., Fraker, T. D., Metting, P. J. and Britton, S. L. (1998). Heritability of treadmill running endurance in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **275**(5), R1455-R1460

Labocha, M.K., Sadowska, E.T., Baliga, K., Semer, A.K. and Koteja, P. (2004) Individual variation and repeatability of basal metabolism in the bank vole, *Clethrionomys glareolus*. *Proc. R. Soc. Lond.* **271**(1537):367-372

Lande, R. and Arnold, S.J. (1983) The Measurement of Selection on Correlated Characters. *Evolution* **37**(6): 1210-1226

Larzul, C., Lefaucheur, L., Ecolan, P., Gogué, J., Talmant, A., Sellier, P., Le Roy, P. and Monin, G. (1997) Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. *J Anim Sci* **75**:3126-3137

Le Conte, Y., Bruchou, C., Benhamouda, K., Gauthier, C. and Cornuet, J.M. (1994). Heritability of the queen brood post-capping stage duration of Apis mellifera mellifera L. *Apidologie* **25**:513-519

Lightfoot, J. T., Turner, M. J., Debate, K. A. and Kleeberger, S. R. (2001). Interstrain variation in murine aerobic capacity. *Medicine and science in sports and exercise* **33**(12), 2053-2057

Lu, Q., Zhong, W.-Q., Wang, D.-H. (2007) Individual variation and repeatability of the aerobic performance in Brandt's voles (*Lasiopodomys brandtii*). *Journal of Thermal Biology* **32**:413-420

Lynch, M. and Walsh, B. (1998) Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc. Sunderland, MA

Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends in Ecology & Evolution* ***6***(6), 183-186

Marden, J.H. (1987) Maximum production during takeoff in flying animals. *Journal of Experimental Biology*. **130**:235-258

McCarthy, I.D. (2000) Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. *Journal of Fish Biology* **57**(1):224-238

Milne Jr, C. P. (1985). A heritability estimate of honey bee hoarding behavior. *Apidologie*, **16**(4), 413-420

Moraes, E.M. and Sene, F.M. (2004) Heritability of wing morphology in a natural population of Drosophila gouveai. *Genetica* **121**(2):119-123

Moritz, RFA (1983) Inbreeding effects in flight muscle mitochondria of *Apis mellifera* L. *Rev. Brasil Genet.* **VI**(1): 59-70

Mousseau, T.D. and Roff., D.A. (1987) Natural selection and the heritability of fitness components. *Heredity* **59**:181-197

Müller, C., Zwaan, B.J., de Vos, H. amd Brakefield, P.M. (2003) Chemical defence in a sawfly: genetic components of variation in relevant life-history traits. *Heredity* **90**:468-475

Naya, D.E. (2010) Why May Repeatability of Highly Flexible Traits Say Little about their Evolutionary Potential? *The Open Ecology Journal* **3***:*26-28

Nespolo, R.F., Bacigalupe, L.D. and Bozinovic, F. (2003) Heritability of energetics in a wild mammal, the leaf-eared mouse (*Phyllotis Darwini*). *Evolution* **57**(7):1679-1688

Nespolo, R.F., Castañeda, L.E. and Roff, D.A. (2007) Quantitative genetic variation of metabolism in the nymphs of the sand cricket, Gryllus firmus, inferred from an analysis of inbred-lines. *Biol Res.***40**(1):5-12

Nespolo, R.F., Franco, M. (2007) Whole-animal metabolic rate is a repeatable trait: a meta-analysis. *Journal of Experimental Biology* **210**(11):2000-2005

Nilsson, J.-A., Äkesson, M. and Nilsson, J.F. (2009) Heritability of resting metabolic rate in a wild population of blue tits. *J Evol Biol.* **22**:1867-1874

Niven, J.E. and Scharlemann, J.P.W. (2005) Do insect metabolic rates at rest and during flight scale with body mass? *Biology Letters* **1**(3):346-349

Oldroyd, B., Rinderer, T., and Buco, S. (1991). Heritability of morphological characters used to distinguish European and Africanized honeybees. *Theoretical and Applied Genetics*, ***82***(4), 499-504

Onyiro, O. M., and Brotherstone, S. (2008). Genetic analysis of locomotion and associated conformation traits of Holstein-Friesian dairy cows managed in different housing systems. *Journal of dairy science*, *91*(1), 322-328

Packard, G. C., and Birchard, G. F. (2008). Traditional allometric analysis fails to provide a valid predictive model for mammalian metabolic rates. *Journal of Experimental Biology*, **211**(22):3581-3587

Parker, W.E. and Gatehouse, A.G. (1985) Genetic factors controlling flight performance and migration in the African armyworm moth, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). *Bull. ent. Res.* **75**: 49-63

Pecsenye, K., Komlosi and Saura, A. (2004) Heritabilities and additive genetic variances of the activities of some enzymes in *Drosophila melanogaster* populations living in different habitats. *Heredity* **93**:215-221

Peters, R. H. (1983). The Ecological Implications of Body Size. Cambridge University Press, New York.

Piiroinen, S., Ketola, T., Lyytinen, A., Lindström, L. (2011) Energy use, diapause behaviour and northern range expansion potential in the invasive Colorado potato beetle. *Functional Ecology* **25**(3):527-536

Poklukar, J., and Kezic, N. (1994). Estimation of heritability of some characteristics of hind legs and wings of honeybee workers (*Apis mellifera carnica Polm*) using the half-sibs method. *Apidologie*, **25**:3-11

Reidy, S.P., Kerr, S.R. and Nelson, J.A. (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. *J Exp Biol.* **203**(2):347-357

Rezende, E.L., Bozinovic, F. and Garland, T. Jr. (2004). Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. *Evolution* **58**(6):1361-1374

Rezende, E.L., Chappell, M.A., Gomes, F.R., Malisch, J.L., Garland, T.Jr (2005) Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running**.** *J Exp Biol* **208**: 2447-2458

Riska, B., Prout, T. and Turelli, M. (1989) Laboratory Estimates of Heritabilities and Genetic Correlations in Nature. *The Genetics Society of America*, 865-871

Riveros, A.J. and Enquist, B.J. (2011) Metabolic scaling in insects supports the predictions of the WBE model. *J Insect Physiol.* **57**(6):688-693

Rogowitz, G.L. and Chappell, M.A. (2000) Energy metabolism of eucalyptus-boring beetles at rest and during locomotion: gender makes a difference. *J Exp Biol.* **203**(7):1131-1139

Rønning, B., Jensen, H., Moe, B. and Bech, C. (2007). Basal metabolic rate: heritability and genetic correlations with morphological traits in the zebra finch. *J. Evol. Biol.* **20**:1815-1822

Rønning, B., Moe, B., Bech, C. (2005) Long-term repeatability makes basal metabolic rate a likely heritable trait in the zebra finch *Taeniopygia guttata.* *J Exp Biol* **208:** 4663-4669

Sacktor, B. (1976) Biochemical adaptations for flight in the insect. *Biochem. Soc. Symp.* **41**:111-131

Sadowska, E.T., Labocha, M.K., Baliga, K., Stanisz, A., Wroblewska, A.K., Jagusiak, W. and Koteja, P. (2005) Genetic correlations between basal and maximum metabolic rates in a wild rodent: consequences for evolution of endothermy. *Evolution* **59**(3): 672-681

Savage, V. M., Gillooly, J. F., Woodruff, W. H., West, G. B., Allen, A. P., Enquist, B. J. and Brown, J. H. (2004). The predominance of quarter-power scaling in biology. *Functional Ecology* **18**: 257–282

Schumacher, P., Weber, D.C., Hagger, C. and Dorn, S. (1997) Heritability of flight distance for *Cydia Pomonella*. *Entomologia Experimentalis et Applicata* **85**:169-175

Skandalis, D.A., Roy, C. and Darveau, C.-A. (2011) Behavioural, morphological, and metabolic maturation of newly emerged adult workers of the bumblebee, Bombus impatiens. *J. Insect Physiol.* **57**(6):704-711

Skandalis D.A., Darveau C.-A. (2012) Morphological and physiological idiosyncrasies lead to interindividual variation in flight metabolic rate in worker bumblebees (*Bombus impatiens*) *Physiological and Biochemical Zoology* **85**(6):657-70

Somero, G.N. and Childress, J. J. (1990) Scaling of ATP-Supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: relationship to locomotory habit. *J. Exp. Biol.* **149**: 319-333

Sorci, G., Swallow, J. G., Garland Jr, T., and Clobert, J. (1995). Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara*. *Physiological Zoology* **68**(4) 698-720.

Stanimirović, Z., Stevanović, J., Aleksić, N., and Stojić, V. (2010). Heritability of grooming behaviour in grey honey bees (Apis mellifera carnica). *Acta veterinaria* **60**(2-3), 313-323

Suarez, R.K. (2000) Energy metabolism during insect flight: Biochemical design and physiological performance. *Physiological and Biochemical Zoology* **73**(6):765-771

Suarez, R. K., Darveau, C.-A., Welch, K.C. Jr, O'Brien, D.M., Roubik, D.W. and Hochachka, P. W. (2005) Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *J. Exp. Biol.* **208**: 3573-3579

Swallow, J. G., Carter, P. A., and Garland Jr, T. (1998). Artificial selection for increased wheel-running behavior in house mice. *Behavior genetics* **28**(3), 227-237

Tanaka, K. (2009) Genetic variation in flight activity of *Ophraella communa* (Coleoptera: Chrysomelidae): Heritability estimated by artificial selection. *Environ. Entomol.* **38**: 266-273

Terblanche, J.S. and Anderson, B. (2010) Variation of foraging rate and wing loading, but not resting metabolic rate scaling, of insect pollinators. *Naturwissenschaften* **97**:775–780

Terblanche, J.S., Klok, J.S. and Chown, S.L. (2004) Metabolic rate variation in Glossina pallidipes (Diptera : Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology* **50**(5):419-428

Tieleman, B.I., Versteegh, M.A., Fries, A., Helm, B., Dingemanse, N.J., Gibbs, H.L. and Williams, J.B. (2009) Genetic modulation of energy metabolism in birds through mitochondrial function. *Proc. R. Soc.* **276**:1685-1693

Tepedino, V. J., Thompson, R., and Torchio, P. F. (1984). Heritability for size in the Megachilid bee *Osmia lignaria propinqua Cresson*. *Apidologie* **15**(1), 83-88

Tsuji, J. S., R. B. Huey, F. H. Van Berkum, T. J. Garland, and R. G. Shaw. **(**1989). Locomotor performance of hatchling Fence Lizard (*Sceloporus occidentalis*): quantitative genetics and morphometric correlates. *Evolutionary Ecology* **3**:240–252

Valera, M., Galisteo, A. M., Molina, A., Miró, F., Gómez, M. D., Cano, M. R., and Agüera, E. (2008). Genetic parameters of biokinematic variables of the trot in Spanish Purebred horses under experimental treadmill conditions. *The Veterinary Journal* **178(**2), 219-226

Van Berkum, F.H., Huey, R.B., Tsuji, J.S. and Garland, T.Jr. (1989) Repeatability of Individual Differences in Locomotor Performance and Body Size During Early Ontogeny of the Lizard *Sceloporus Occidentalis* (Baird & Girard). *Functional Ecology* **3**(1):97-105

Van Dyck, H., Matthysen, E., Wiklund, C. (1998) Phenotypic variation in adult morphology and pupal colour within and among families of the speckled wood butterfly *Pararge aegeria*. *Ecological Entomology* **23**(4):465-472

Virani, N.A., Rees, B.B. (2000) Oxygen consumption, blood lactate and inter-individual variation in the gulf killifish, Fundulus grandis, during hypoxia and recovery. *Comparative Biochemistry and Physiology* **126**(3):397-405

Vogt, F.D., Heinrich, B. and Plowright, C. (1998)Ovary development in bumble bee queens: the influence of abdominal temperature and food availability**.** *Canadian Journal of Zoology* **76 (11)**: 2026-2030

Watt, W.B., Wheat, W., Meyer, E.H. and Martin, J.-F. (2003) Adaptation at specific loci. VII. Natural selection, dispersal and the diversity of molecular–functional variation patterns among butterfly species complexes (*Colias*: Lepidoptera, Pieridae). *Molecular Ecology* **12**, 1265–1275

Weigensberg, I. and Roff, D. (1996) Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution* **50 (6)**: 2149-2157

White, C. R., and Seymour, R. S. (2005). Allometric scaling of mammalian metabolism. *Journal of Experimental Biology* **208**(9), 1611-1619

Whitehorn, P. R., Tinsley, M. C., Brown, M. J., Darvill, B., and Goulson, D. (2009). Impacts of inbreeding on bumblebee colony fitness under field conditions. *BMC evolutionary biology* **9**(1): 152

Zakour, M. K., Ehrhardt, K., and Bienefeld, K. (2012). First estimate of genetic parameters for the Syrian honey bee *Apis mellifera syriaca*. *Apidologie*, ***43***(5), 600-607

Zub, K., Piertney, S., Szafranska, P.A. and Konarzewski, M. (2012) Environmental and genetic influences on body mass and resting metabolic rates (RMR) in a natural population of weasel *Mustela nivalis*. *Molecular Ecology* **21**(5):1283-1293