

# Sex, life history and morphology drive individual variation in flight performance of an insect parasitoid

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**Abstract.** 1. The movement of organisms can be driven by multiple factors and has implications for fitness and the spatial distribution of populations. Insects spend a large proportion of their adult lives foraging by flying for resources; however, their capability and motivation to move can vary across individuals.

2. The aims of this study were to examine interindividual and sex differences in flight performance and flight characteristics, using a flight mill bioassay, in *Megarhyssa nortoni* (Hymenoptera; Ichneumonidae), a parasitoid of the invasive woodwasp *Sirex noctilio* (Hymenoptera: Siricidae), one of the most important pests of pine afforestation worldwide. We also assessed the influence of morphological traits in combination with sex on flight and explored the cost of flight on longevity and mass loss.

3. The results show a difference between sexes in flight characteristics and performance. Females show greater total distance flown than males, and have a better capacity to undergo sustained flight. Sexual size dimorphism was also found and it was noted that size positively affects distances travelled. Females have a longer life span than males, yet no differences were noted in longevity within sex between individuals that did not fly and those that flew. Age did not influence flight performance of females or impacted on post-flight longevity. Females lost less body mass than males even after flying longer distances.

4. These results suggest that sex-specific behaviours probably govern flight abilities together with (and not only because of) morphological traits. The paper discusses sex-specific life-history strategies in parasitoids and their implications for biocontrol programmes.

**Key words.** Flight mills, flight morphological traits, flight patterns, insect movement, *Megarhyssa nortoni*, parasitoids.

## Introduction

The movement of organisms in space has direct implications for individual fitness and determines the genetic composition and dynamics of natural populations. Whether passive or active, the capability of individuals to move depends on multiple causal factors, such as their internal state or the physical and biotic conditions of the environments in which they dwell (Bowler & Benton, 2005; Benton & Bowler, 2012; Matthysen, 2012).

The life history and the sex of the organisms are also important in determining where and when to move (Bowler & Benton, 2005). Females and males may differ in several behaviours or the degree to which they fulfil a given behaviour. In insects, sex-biased flight capacity can be the result of body size polymorphism and/or wing polymorphism – the latter, when one of the sexes has fully developed wings while the other has no wings or reduced size wings (Zera & Denno, 1997; Chapman, 2007). In addition, for winged organisms, variations in flight performance might be the consequence of variations in morphological traits or suites of traits. This issue has primarily been studied in birds (Liu & Zhang, 2008), and, among insects, in butterflies (Berwaerts *et al.*, 2002, 2006).

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In parasitoids – those insects that lay eggs in or on other arthropods, eventually killing them – movement by flight is crucial in population redistribution and in foraging for resources required for survival and reproduction, i.e. food, mating partners, oviposition sites or hosts (Bowler & Benton, 2005; Hardy *et al.*, 2005). Importantly, parasitoid females and males can move for different reasons. While male parasitoids often spend most of their flight time locating mates, females mostly do so in the search of host habitats and host patches on which to oviposit. These sex-specific life-history strategies probably require different flight abilities and behaviours, which may be linked to differences in flight morphological traits between sexes.

Variation in flight performance between individuals can be based on differences in life-history trade-offs (Zera & Denno, 1997; Zera & Harshman, 2001; Hanski *et al.*, 2006; Guerra, 2011). Active flight is considered to be a costly metabolic activity that can constrain other biological functions such as longevity and reproduction (mainly in females) by means of draining resources (Hanski *et al.*, 2006; Elliott & Evenden, 2012). How an individual manages its energy budget should be expressed in interindividual differences in flight performance (Jervis *et al.*, 2008). Likewise, a relationship between age and movement ability can be determined by different constraints or costs associated with the displacement (Bowler & Benton, 2005). In parasitoid wasps, for example, demands for moving by flight to find oviposition sites, food or mates, are likely to affect different age classes differently. This is because ecological needs associated with reproduction can be age-specific and so may drive movement accordingly. At the same time, the underlying temporal variation in their physiological status will affect the strength of pressures behind movement.

In general, insect flight encompasses a wide range of movements from relatively short displacements, such as those displayed during foraging bouts, to very long dispersal distances or migration. Besides the effects of displacement on the spatial distribution of a species, the movement of individuals can play a central role in the stability and persistence of species interactions, as has been noted for host–parasitoid systems (Hassell & May, 1973; Roland & Taylor, 1997). For pest natural enemies such as parasitoids released in biocontrol programmes, flight capacity can be critical to their success. Species with a high flight capability may disperse well from the release sites and quickly colonize isolated host patches where no releases have been deployed (Kidd & Jervis, 2007). In contrast, overdispersal mediated by a tendency to fly long distances or a strong differential response in flight displayed by males and females can decrease the chances of establishment of new populations, increasing the likelihood of Allee effects (Heimpel & Asplen, 2011).

The present study focuses on the flight performance of *Megarhyssa nortoni* (Cresson) (Hymenoptera; Ichneumonidae), and its consequence on longevity and body mass loss. *Megarhyssa nortoni* is a parasitoid native from North America that was introduced into Australia, New Zealand, and South America as a biological control agent against the invasive woodwasp *Sirex noctilio* (Hymenoptera: Siricidae), probably most important pests of pine afforestation in the southern hemisphere (Cameron, 2012). We hypothesise that

sex-specific life-history strategies should be associated with different flight capacities and characteristics that probably lead to differential adult life physiological and ecological costs. Specifically, this study aims to: (i) examine sexual differences in flight performance; (ii) examine the influence of morphological traits in combination with sex on flight; (iii) assess the effect of age on female flight performance; and (iv) study the costs of flight activity on post-flight longevity and body mass loss after flight. This analysis can shed light on the interrelations between morphology and flight in parasitoid wasps. Lastly, due to the fact that female movements largely determine the spatial distribution of their progeny, a greater knowledge of potential flight capacity of *M. nortoni* might allow us to gain further insights into the potential expansion of this parasitoid species wherever it is released in biocontrol programmes.

## Materials and methods

### *Insect collection*

*Megarhyssa nortoni* (Cresson, 1864) (Hymenoptera: Ichneumonidae) is a solitary, idiobiont, ectoparasitoid of siricid larvae. For this study, adults of *M. nortoni* were obtained from *Sirex*-infested pine logs (*Pinus contorta*) collected in the field from several heavily attacked plantations located in northwest Patagonia (Argentina). Once felled, trees were cut into 1-m-long logs and kept in cages under semi-controlled ambient conditions (21 °C) until adult emergence. Daily, newly emerged adult wasps were collected from the cages and immediately placed in individual plastic vials with water for use in experimental treatments.

### *Flight performance measurement*

In order to quantify adult flight performance for both sexes, 1-day-old females ( $n = 41$ ) and males ( $n = 39$ ) were tethered to a fixed-arm, processor-linked flight mill under controlled temperature ( $23 \pm 1$  °C), RH (45%) and LD 16:8 h. To test whether there is age-dependent variation in flight performance, we also measured the flight of 10-day-old females ( $n = 19$ ) in the flight mills. We carried out this objective in females only, because of the greater putative relevance of active movements to population dispersal and spread. It has been suggested that, for this parasitoid, 10 days old represents an advanced age, taking into account preliminary data on female life expectancy in laboratory conditions (Fischbein, unpublished). Males and females were flown on separate days over several weeks. This was based on the availability of experimental wasps. To determine whether flight activity impacted on post-flight longevity and body mass loss after flight, females and males that were flown at 1 day old were then kept in plastic containers (1000 cm<sup>3</sup>) covered with chiffon-type cloth in a controlled climate chamber (temperature  $23 \pm 1$  °C, RH 45% and LD 16:8 h) until they died. Control females ( $n = 34$ ) and males ( $n = 46$ ) – those that were not tethered in flight mills – were kept under the same conditions. All wasps were provided with distilled water and checked daily.

**Table 1.** Measured and derived flight variables extracted from flight mill data used in this survey to study flight performance.

Flight variables	Definitions
Total distance flown (m)	Cumulative sum of the distances flown in the whole trail
Number of single flights	Count of flights
Average single-flight distance (m)	Mean distance of single flights
Average single-flight duration (s)	Mean duration of single flights
Average single-flight speed ( $\text{m s}^{-1}$ )	Mean speed of single flights
Furthest single-flight distance (m)	Distance travelled in the single flight of greatest distance
Longest single-flight distance (m)	Distance travelled in the single flight of greatest duration
Longest single-flight duration (s)	Duration of the single flight of greatest duration

Flight mills used in this study were similar in structure to those used in Bruzzone *et al.* (2009). However, the current mills have magnetic sensors. These sensors located on each mill allowed us to register in a computer each half rotation of the mill arm by means of a signal. Hence, the computer recorded each spin as the wasp propelled the wooden rod arm which was 20 cm long. Flight trials began between 4 and 5 h after the onset of the photo-phase. Each individual flew on the mills for 22 h and was weighed immediately before and after flight (Scientech Inc., Boulder, Colorado; 0.0001 g). Wasps were anaesthetised with  $\text{CO}_2$  for  $\approx 30$  s (Fischbein *et al.*, 2011) and the dorsal side of the thorax was glued to one end of an insect pin, using cyanoacrylate glue (La Gotita, Poxipol, Buenos Aires, Argentina). The pin was shaped by hand into an L shape, in order to create a greater surface area of contact between the pin and the insect thorax. Finally, the opposite (free) end of the pin was attached to one end of a wooden, horizontal rod which acted as the mill axis. Wasps were left to recover from the  $\text{CO}_2$  treatment for  $\approx 15$  min before flight tests began. We chose to fly insects for 22 h because we considered that this period was long enough to allow us to infer maximum potential flight capacity of the parasitoid throughout a day (i.e. displaying a daily activity rhythm), as well as to evaluate the consequences of flight on some of the studied parameters (body mass loss and longevity).

Raw data recorded by the flight mills were as follows: the number of revolutions, the cumulative sum of the distances flown (m), the cumulative sum of time spent flying (s), and flight speed ( $\text{m s}^{-1}$ ). These flight data for each individual wasp were then processed to calculate the derived flight variables (described in Table 1) using a specific script written in R language (R Development Core Team, 2016). In essence, the sequence of revolutions was interpreted in terms of single-flight bouts (SF) and stops. A stop was defined as a time period with extremely slow revolutions (flight speed  $< 0.01 \text{ m s}^{-1}$ ) and a flight bout (henceforth, single flight) as any flight period between stops. Hence, flight performance was measured as the total distance flown and the single flight variables, which may reflect interindividual variation in flight behaviour. Only wasps that flew more than 100 m were included in the statistical analysis. We supposed arbitrarily that those that flew less ( $n = 10$ ) during the 22 h period were unresponsive.

#### Adult morphological variables

Several adult morphological variables were measured to determine whether morphological variation leads to variation in

flight performance. As indicators of size, we measured hind tibia length (TL), forewing length (FWL) and body mass at emergence (BME); as an indicator of flight muscle investment we measured thorax size (TS); and as an indicator of flight loading capacity and wing shape we measured wing loading ( $\text{WL} = \text{body mass}/\text{total wing area}$ ) and aspect ratio ( $\text{AR} = 4 \times \text{forewing length}^2/\text{forewing area}$ ; Berwaerts *et al.*, 2002), respectively. Total wing area (TWA = sum of forewing and hindwing area) and FWL were measured from photographs of wings, mounted on microscope slides, taken by an Infinity 1 digital camera (Lumenera Corp., Bothell, Washington) mounted on a Olympus SZ61 stereo microscope  $0.67\times$  (Olympus Corp., Tokyo, Japan), and processed with IMAGEJ (Rasband, 2014). The TL and TS were measured under a binocular lens with a digital caliper. These traits have often been studied in relation to animal flight (Dudley, 2002).

#### Statistical analysis

Generalised linear models (GLMs) with a Gamma distribution and log link function were used to determine the effect of sex on the different flight parameters. In addition, several separate analyses were done to test the relationship between flight performance variables and flight morphology traits (BME, TL, TS, FWL, TWA, WL, AR) in combination with sex, by including interaction terms (Tables S1–S4). Non-significant terms were removed from the model in a stepwise manner until the most parsimonious model remained. The significance of each effect term was assessed by comparing the deviances of models fitted with and without each effect term by  $\chi^2$  tests. Female wasps were used to test whether there is age-dependent variation in flight performance. A GLM was also fitted with a Gamma distribution and log link function, with all the flight parameters as the dependent variable and age as the explanatory variable (1 day old and 10 days old, i.e. early flight and late flight, respectively). To determine whether flight activity impacted on female and male longevity, a Cox proportional hazards model for survival data was used. First, longevity was used as dependent variable and sex or age as explanatory variables. Then, morphological traits were also added as explanatory variables (i.e. BME, TS and TL) (Table S5). To study the effect of total distance flown in combination with age (two levels) on relative mass loss (RML) in females, a linear model was used. RML was estimated as (mass prior to flight – mass post-flight)/BME. To study the effect of total distance flown in

combination with sex on RML, a linear model was used with RML log-transformed. In all cases, residuals were examined and all analyses were done using the R statistical environment (R Core Development Team, 2016).

## Results

Our results show significant differences among sexes of the parasitoid *Megarhyssa nortoni* in several of the flight performance variables estimated (Fig. 1), with adult females tending to display a better overall performance than males. The maximum recorded total distance flown by one female was 36 175 m in a total of nine single flights over the 22-h period, while the maximum for a male was 11 808 m in a total of 11 single-flight bouts. Interestingly, females had a better capacity to undergo sustained flight, as measured by the furthest single-flight distance and longest single-flight duration recorded (mean  $\pm$  SE; furthest SF distance: female, 2658.52  $\pm$  793.89 m; male, 911.53  $\pm$  264.18 m; longest SF duration: female, 3049.06  $\pm$  807.27 s; male, 1176.65  $\pm$  279.64 s) – thus, almost a threefold difference and more than a twofold difference between females and males for these variables, respectively.

Sexual differences in morphological traits are summarised in Table 2. Females are, on average, larger than males. When analysing to what extent the significant differences in flight performance variables between sexes could be explained by morphological traits in combination with sex, we noted that there was no significant effect of the interaction between sex and each morphological trait on any of the flight variables studied. However, except for aspect ratio, which had no effect on flight variables, flight performance parameters generally increased with increased value in the morphological traits evaluated here (see Tables S1–S4).

Contrary to our expectations, when comparing females flown at two different ages (1 and 10 days) these did not show significant differences in any of the flight variables studied, although there is a tendency for them to decrease in older insects (Table 3). In addition, flight activity did not impact on longevity of female *M. nortoni* wasps. The longevity of females that did not fly was similar to that of females that flew either early or late in life ( $z = 0.588$ ,  $P = 0.556$ ; and  $z = 0.428$ ,  $P = 0.669$ , respectively). In the same manner, there was no difference in longevity between females that flew earlier and those that flew later in their lifetime ( $z = 0.037$ ,  $P = 0.970$ ; Table 4). Finally, we did observe significant differences in longevity between males and females ( $z = 4.543$ ,  $P < 0.0001$ ). Females have a longer life span than males having flown or not (Table 4). Additionally, no difference in longevity was found within sex between individuals that were not tethered in the flight mills (control) and those that flew ( $z = 0.951$ ,  $P = 0.342$ ). No effects were observed for the interaction between sex and flight ( $z = -0.451$ ,  $P = 0.652$ ).

When the effect of sex and size on longevity was studied by including TS, TL or BME in the Cox proportional hazards models, we found a significant effect of the interaction among sex, size and flight on longevity, specifically, in the case where body mass was used as size variable ( $z = 2.152$ ,  $P = 0.031$ ; Fig. 2). In addition, the variability in longevity could be explained mainly by size and not exclusively by gender (see Table S5).

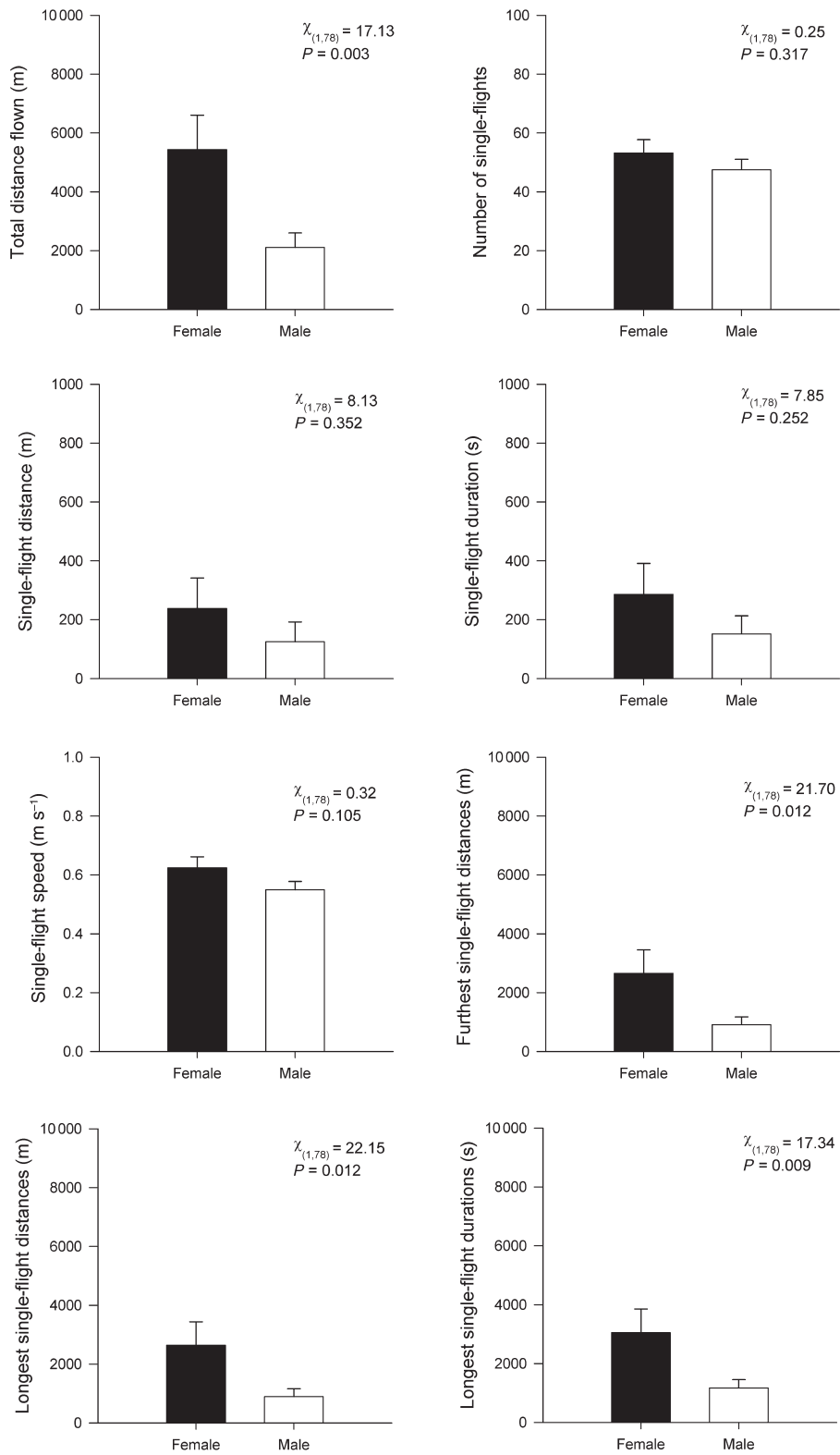
Results of the analysis of the impact of flight activity on RML in female wasps showed that females that flew earlier had a significantly greater loss of body mass than those that flew later in their lifetime (mean  $\pm$  SE;  $RML_{\text{early-flight}}$ , 0.107  $\pm$  0.004 g,  $n = 41$ ;  $RML_{\text{late-flight}}$ , 0.083  $\pm$  0.006 g,  $n = 19$ ;  $F_{1,58} = 9.513$ ,  $P_{\text{age}} < 0.01$ ), irrespective of the total distance flown ( $F_{1,57} = 0.0480$ ,  $P_{\text{total distance flown}} = 0.827$ ). Additionally, the impact of flight on RML was also significantly greater for males than for females ( $RML_{\text{males}}$ , 0.140  $\pm$  0.006 g,  $n = 40$ ;  $RML_{\text{females}}$ , 0.107  $\pm$  0.004 g,  $n = 41$ ;  $F_{1,75} = 15.595$ ,  $P_{\text{sex}} < 0.0001$ ), irrespective, again, of the total distance flown ( $F_{1,74} = 1.409$ ,  $P_{\text{total distance flown}} = 0.238$ ).

## Discussion

Movement by flight plays a very important role in many insects because these animals spend a large proportion of their adult lives foraging for what are widespread resources relative to their body size. Several empirical studies in insects have shown how flight performance and the potential capacity to fly are influenced by morphology as well as endogenous and environmental factors (Sarvary *et al.*, 2008; Heimpel & Asplen, 2011; Benton & Bowler, 2012; Kisdi *et al.*, 2012; Zboralski *et al.*, 2016). For parasitoids, flight movements may lead to successful host encounter and attack, which translates directly into fitness. In our study, we show that sex, body and wing size explain the variability recorded in flight performance and characteristics among individuals of the parasitoid *M. nortoni*. In accordance with our expectations, females showed a greater total distance flown than males (i.e. flight capacity), and they were also able to sustain longer single flights (i.e. flight characteristics).

The greater capacity of females for sustained flight may well be based on a sex-specific resource availability and allocation to locomotion. Arising from sex-specific life-history strategies, the putative differential use of resources between sexes can be an adaptive response to the fact that female parasitoids are responsible for colonising new habitats, locating suitable hosts and redistributing the progeny, activities that all frequently involve longer-range movements. Female-biased flight behaviour has been already suggested for other parasitoid wasp species (Ode *et al.*, 1998; Bellamy & Byrne, 2001). We show this is also true for *M. nortoni*, a species that undergoes solitary larval development and so needs to locate a new individual host for each oviposition.

For their part, the life-history strategy of *Megarhyssa* male wasps should be different from that of females, by having different ecological roles and evolutionary constraints. Males spend a large proportion of their time in aggregations on female emergence sites at trees, waiting for them to appear to mate (Nuttall, 1973; Matthews *et al.*, 1979; Crankshaw & Matthews, 1981). These aggregations are persistent to the point that if males are disturbed, they may fly away but then regroup within the same spot on the tree. Moreover, it has been noted that males show a high fidelity to a particular tree, returning to patrol on it over time (this would be favoured by a presumed trails laid by males; Matthews *et al.*, 1979). This tree fidelity, together with



**Fig. 1.** Effect of sex on flight performance variables estimated (mean  $\pm$  SE) in the parasitoid wasps of *Megarhyssa nortoni* flown in flight mills early in adult life. Sample size: females,  $n = 41$ ; males,  $n = 39$ .

**Table 2.** Comparison of flight morphological traits between sexes of *Megarhyssa nortoni* parasitoid wasps.

Morphological traits	Sex		<i>t</i> -Test		
	Females ( <i>N</i> = 61) Mean ± SE	Males ( <i>N</i> = 52) Mean ± SE	<i>t</i>	d.f.	<i>P</i> -value
Weight at emergence (g)	0.32 ± 0.02	0.12 ± 0.006	11.12	111	<0.0001
Tibia length (mm)	8.40 ± 0.18	6.11 ± 0.13	10.26	111	<0.0001
Thorax size (mm)	4.00 ± 0.08	2.83 ± 0.065	10.79	111	<0.0001
Forewing length (mm)	22.27 ± 0.46	16.11 ± 0.30	11.02	111	<0.0001
Total wing area (mm <sup>2</sup> )	257.51 ± 10.00	151.05 ± 5.34	9.35	111	<0.0001
Wing loading (g mm <sup>-2</sup> )	0.0012 ± 0.00002	0.0008 ± 0.00002	12.98	111	<0.0001
Aspect ratio <sup>†</sup>	24.45 ± 0.15	21.812 ± 0.09			<0.0001

<sup>†</sup>Wilcoxon rank sum test. *W* = 3099, *P* < 0.0001.

Means (± SE) shown are based on untransformed data. *t*-Tests were done on untransformed and log-transformed data when appropriate (i.e. body mass at emergence, forewing length, total wing area).

**Table 3.** Summary of flight performance parameters (mean ± SE) for females of different ages (1 and 10 days old).

Flight performance parameters	Early-flight females ( <i>n</i> = 41)		Late-flight females ( <i>n</i> = 19)		$\beta$	Statistic	<i>P</i>
	Mean	±SE	Mean	±SE			
Total distance flown (m)	5435.08	1168.89	4056.9	1116.11	-0.29	$\chi_{(1,58)} = 1.06$	0.43
Number of single flights	53.19	4.52	44.31	5.78	-0.18	$\chi_{(1,58)} = 0.42$	0.23
Single-flight distance (m)	238.72	103.19	195.51	75.30	-0.19	$\chi_{(1,58)} = 0.50$	0.77
Single-flight duration (s)	285.780	105.46	321.404	126.38	0.11	$\chi_{(1,58)} = 0.18$	0.84
Single-flight speed (m s <sup>-1</sup> )	0.62	0.03	0.55	0.03	-0.12	$\chi_{(1,58)} = 0.20$	0.19
Furthest single-flight distances (m)	2658.52	793.89	1993.88	606.91	-0.28	$\chi_{(1,58)} = 1.03$	0.56
Longest single-flight durations (s)	3049.06	807.27	2943.45	858.95	-0.03	$\chi_{(1,58)} = 0.01$	0.93
Longest single-flight distances (m)	2645.43	794.92	1992.53	607.07	-0.28	$\chi_{(1,58)} = 1.00$	0.56

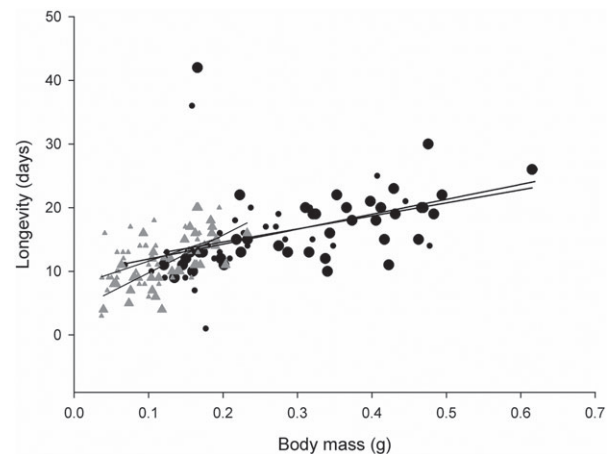
**Table 4.** Longevity of *Megarhyssa nortoni* adults for both sexes (days).

Treatment	<i>n</i>	Mean ± SE
Females		
Early flight	41	17 ± 0.97 a
Late flight	19	17.15 ± 0.94 a
No flight	34	15.44 ± 1.04 a
Males		
Early flight	39	11.48 ± 0.67 b
No flight	46	11.67 ± 0.49 b

Significantly different flight treatments are indicated with different letters (a, b).

the time spent patrolling female emergence sites, could imply a need for short-range movement skills through their adult life.

Interindividual differences in behavioural performance can result from continuous variations in morphological traits (Berwaerts *et al.*, 2002, 2006). Our results show that both a sexual size dimorphism and adult size affect flight distances travelled. The variation in flight performance was positively related to variations in morphological traits in both sexes; yet there is no significant effect of the interaction between sex and morphological traits on flight. This is in accordance with the growing evidence in many taxa that individuals that display greater capacity to move long distances, mainly by flying, are larger and perhaps in a better condition to bear the costs of moving (insects: Anholt, 1990; Bruzzone *et al.*, 2009; Fischbein *et al.*, 2011; birds:

**Fig. 2.** Longevity as a function of body mass for females flown early in life (large black circle), females not flown (small black circle), males flown early in life (large grey triangle circle), and males not flown (small grey triangle). See also Table S5.

Barbraud *et al.*, 2003; Liu & Zhang, 2008). Long-distance movements are considered to be energetically expensive and may also involve a high risk of mortality (Bonte *et al.*, 2012). Therefore, those individuals with sufficient energy reserves would have better chances to survive. It should be noted, however, that the effects of phenotypic quality on the propensity to

move may involve different mechanisms, and so it is not always those individuals in better condition that are more likely to move (in some taxonomic groups high individual quality condition may imply both high competitive ability and low propensity to move from natal environment; Clobert *et al.*, 2009; Matthysen, 2012).

Regarding body design in relation to flight, it is thought that low wing loading decreases the cost of flight movements and that high wing loading is associated with the need to carry adequate fuel supplies (Yong & Moore, 1994; Bowlin & Wikelski, 2008). Here, we found that females of *M. nortoni* have a greater wing area than males; however, this is not accompanied by a lower wing loading for females in comparison with males as females also have a greater body mass. Therefore, it appears that flight would not be less energetically expensive for parasitoid females than for males; rather, female wasps would carry more reserves with them to fuel sustained flight.

Age-dependent variation in flight movement and capacity can be a recurrent pattern attributed to species-specific temporal changes in physiological status (Schumacher *et al.*, 1997; Elliott & Evenden, 2009; Lukás *et al.*, 2010). However, this is not a rule for all parasitoid species. For example, flight duration of *Nasonovia vitripennis* differs slightly between young and old individuals (King, 1993). In the present study, 10 day-old females showed similar flight capacities to 1-day-old females. Note that 10-day-old females lie above the middle range of the estimated female life span (see Table 4). Therefore, it seems that female wasps emerge with sufficient capital energy reserves to allocate to flight such that it is not compromised later in their lifetime. This permanent flight capacity could allow insects to deal with variability in environmental conditions and host availability. It should be noted that these results were obtained by analysing tethered flight; the next step is to confirm these findings in free flight experiments.

Resource availability and body size often determine energy allocation to flight, longevity and reproduction in insects (Shirai, 1995; Zera & Denno, 1997; Jervis *et al.*, 2008; Elliott & Evenden, 2009). In this regard, our results show the lack of effects of age on flight, and of flight at different ages on longevity. These findings suggest that females may emerge as adults with enough capital reserves such that the expected physiological trade-offs between these biological functions is not expressed. Although, the lack of a significant correlation between two traits is insufficient to demonstrate the absence of a trade-off, it still measures, to some degree, the extent to which the two traits can vary independently. Trade-offs usually arise under strong environmental stress, where resources are limited. It is likely that our experiment does not accurately reflect the condition in which *M. nortoni* has evolved, and so the trade-offs we expected to see are not apparent from our results. Further, measuring tethered flight in laboratory conditions has its limitations. Insects are not faced with the full energetic costs of free flight, for instance, as they do not have to deal with wind and they do not have to carry their own body weight. Therefore, the lack of effects of flight on longevity within sexes and of age on flight, as measured in flight mill bioassays, must be interpreted with caution. Flight mills can overestimate or underestimate flight performance.

While we found that females live longer than males, there were no differences within sex between individuals that did and did not fly. Still, results show that life span variability can be explained by size traits in combination with sex. A small increase in body mass results in a greater increase in life span in males than in females. This could be a selective advantage for males whose mate-finding strategy and copulation behaviour at the conspecific emergence sites may be considerably time-consuming, given their limited ability to discriminate the sex of the emerging adult before these surface (Crankshaw & Matthews, 1981). In this way, time could be more limiting for males than for females. Males would need additional time to compensate for the time spent waiting at aggregations over the 'wrong' emergence sites. Therefore, males could have evolved to achieve greater longevity with small increases in body mass.

Regarding the analysis of the cost of flight activity measured as relative body mass loss by individuals, we can see how a slightly inferior (though not significant) flight performance by older females may bring about a significantly lower RML for the older than for the younger wasps. Additionally, it appears that females are more efficient in their use of energy than males. This is because females lose a smaller proportion of body mass than males even when flying longer distances. As we noted previously, female parasitoids are commonly responsible for activities that frequently involve longer-range movements and this could drive them towards an efficient use of their energy budget. A full understanding of these findings requires further research to provide empirical evidence of resource allocation (e.g. lipids, amino acids, carbohydrates) involved in flight and survival.

### Conclusions

Movement allows for the redistribution of individuals, links together suitable locations for foraging, and determines the spatial structure of animal populations. In parasitoids, movement is essential to reproduce and hence is directly related to fitness. It is also key to the success of biological control programmes. Insect flight and how it relates to life-history traits can vary between individuals, age groups, and sexes. For parasitoids in general, and *M. nortoni* in particular, assessment of flight potential in a controlled environment, though limited, allows us not only to determine factors that may govern variation in dispersal in nature, but also to improve our insight into the potential of establishment and spatial dynamics of the species. This may be a central point in parasitoid ecology and a key issue when species are used as control agents in pest management programmes.

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Agropecuaria (INTA). The authors have no conflict of interest regarding this publication. Authors' contributions: DF, JCC, and JMV conceived the ideas and designed the methodology; DF collected the data; DF and GV analysed the data; and DF and JCC led the writing of the manuscript. All authors contributed to critical interpretation of the data, revising the draft and gave their final approval for publication. DF and JCC provided the funds and organised the research programme.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12469

**Table S1.** Effect of sex in combination with different size morphological traits on the total distance flown.

**Table S2.** Effect of sex in combination with different size morphological traits on the furthest single-flight distances.

**Table S3.** Effect of sex in combination with different size morphological traits on the longest single-flight distances.

**Table S4.** Effect of sex in combination with different size morphological traits on the longest single-flight durations.

**Table S5.** Effect of sex, size and flight on longevity.

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