



## Viability selection on female fly finery in the wild

DARRYL T. GWYNNE<sup>1</sup>, DAVID PUNZALAN<sup>2</sup> and JOHN HUNT<sup>3</sup>

<sup>1</sup>Biology Dept University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, ON, L5L 1C6, Canada

<sup>2</sup>Royal Ontario Museum, 100 Queen's Park, Toronto, ON, M5S 2C6, Canada

<sup>3</sup>Daphne du Maurier Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, TR10 9FE, UK

Received 11 March 2015; revised 7 May 2015; accepted for publication 8 May 2015

Female ornaments have evolved in a few taxa in which females compete for access to important resources provided by their mates. However, the effects of these sexually selected traits on survival have not been studied. Elaborate leg-scale and/or abdominal ornaments are displayed by females of some *Rhamphomyia* dance flies (Diptera: Empididae) to flying males carrying prey gifts (females do not hunt). Previous analyses have shown significant sexual selection on these female traits. We studied viability selection on the traits by sampling the webs of two spider species and comparing prey *R. longicauda* females to survivors. We also investigated viability selection from one of the spiders over two seasons. We found that the direction of viability selection on *R. longicauda* from sticky *Tetragnatha* spider webs was consistent over two seasons. For abdominal ornaments the form of viability selection was positive and primarily directional (linear). Viability selection also favoured shorter tibiae but there was no significant selection on the size of residual tibial scale area. However, with the addition of dance fly kills from the non-sticky, leaf-covering webs of a *Dictyna* spider, abundant in only one of the seasons, the overall direction of viability selection favoured larger tibial ornaments. While noting that this viability selection on tibial scale ornaments may be a statistical artefact of the fewer traits in the two-predator analysis (abdominal structures were missing from most *Dictyna* prey), we suggest that simple differences in the natural history of selective agents causing mortality may partly explain the variation in whether sexual traits are under viability selection. Viability selection on ornamental traits may vary greatly between seasons with changes in the abundances of different natural enemies so that net directional selection on these traits over many generations may be weak. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, 116, 530–540.

**ADDITIONAL KEYWORDS:** female ornaments – role reversal – sexual dimorphism – sexual selection – spider predation.

### INTRODUCTION

Ornaments and armaments are typically thought to have survival costs (Darwin, 1871; Fisher, 1930; Andersson, 1994), although opposing theories (Wallace, 1889) including ‘indicator mechanism’ models (e.g. Grafen, 1990) have associated the expression of ornaments with increased viability (Cronin, 1991). Survival is often assessed in cage and semi-natural enclosure studies of predation risk (Kotiaho *et al.*, 1998; Hernandez-Jimenez & Rios-Cardenas, 2012) or longevity (e.g. Hunt *et al.*, 2004; Judge, Ting &

Gwynne, 2008), but a full understanding of the relationships between sexually selected traits and survival examines populations in the wild exposed to natural levels and types of mortality (e.g. Quinn, Hendry & Buck, 2001; Robinson *et al.*, 2006; Ercit & Gwynne, 2015). Also, in order to understand the temporal patterns of selection over time, studies over several seasons are important (Siepielski, DiBattista & Carlson, 2009; Siepielski *et al.*, 2011; Morrissey & Hadfield, 2012). Research to date has focussed on viability associated with male traits, probably because sexually selected female traits (those associated with mating success: Shuker, 2010) occur in fewer taxa. Female-specific ornaments are found in

\*Correspondence author. E-mail: darryl.gwynne@utoronto.ca

species in which females compete to mate with males supplying food gifts or valuable care to offspring (Gwynne, 1991; Tobias, Montgomerie & Lyon, 2012). In a few insects, female ornaments are displayed to gift-giving males (but see South & Arnqvist, 2011) and include the wing patterns and coloration in butterflies (Oliver, Robertson & Monteiro, 2009; Tigreros, Mowery & Lewis, 2014) as well as elaborate leg scales, inflated abdomens and wing coloration in many empidine dance flies (Empididae: Richards, 1927; Downes, 1970; Cumming, 1994).

Empidines use gifts of prey. In *Empis* and *Rhamphomyia*, females do not hunt, and instead rely on prey provided by males (Downes, 1970). In some species this action results in sexual competition between females within lek-like swarms (Svensson & Petersson, 1987; Funk & Tallamy, 2000; LeBas, Hockham & Ritchie, 2003), with Cumming (1994) suggesting that such reversed sexual selection may be frequent in empidines given that about 25% of the hundreds of *Empis* and *Rhamphomyia* species show female-specific ornamentation. Inflated abdomens and/or outstretched scale-covered legs appear to be displays that enhance the size of the slow moving females within all-female swarms (Newkirk, 1970; Cumming, 1994).

Field studies of sexual selection on female dance flies began with behavioural studies of male choice and female ornaments (Svensson & Petersson, 1988; Svensson, Petersson & Forsgren, 1989; Funk & Tallamy, 2000) and were followed by sexual selection analyses of the ornaments. LeBas *et al.* (2003) showed nonlinear (roughly quadratic) sexual selection in *Rhamphomyia tarsata* for increased size of the tibial scale ornaments of females (body size controlled). For *R. longicauda*, Wheeler, Gwynne & Bussière (2012) also found quadratic selection supporting the hypothesis (Chenoweth, Doughty & Kokko, 2006) that female (tibial scale and inflated abdomen) ornaments were under stabilizing sexual selection.

Evidence that sexual traits of females increases their risk of predation is that displaying *R. longicauda* females are caught more often in spider webs than males (Gwynne & Bussière, 2002) due to a sex bias in risk of entanglement (Gwynne, Bussière & Ivy, 2007). Moreover, these experiments using webs from two spider species (Gwynne *et al.*, 2007) showed a trend for a greater risk of inflated females than uninflated females to become entangled.

There is a need for field studies to understand how survival selection acts on traits under sexual selection (Kingsolver *et al.*, 2001; Svensson & Gosden, 2007; Siepielski *et al.*, 2011), especially in females as studies are lacking. In this study, we investigated viability selection in the wild on the female ornaments of *R. longicauda*, using selection analyses to

compare traits of web prey from two spider species (Fig. 1) to traits of surviving flies. For one of the predators we compare viability selection on female traits over 2 years.

## METHODS

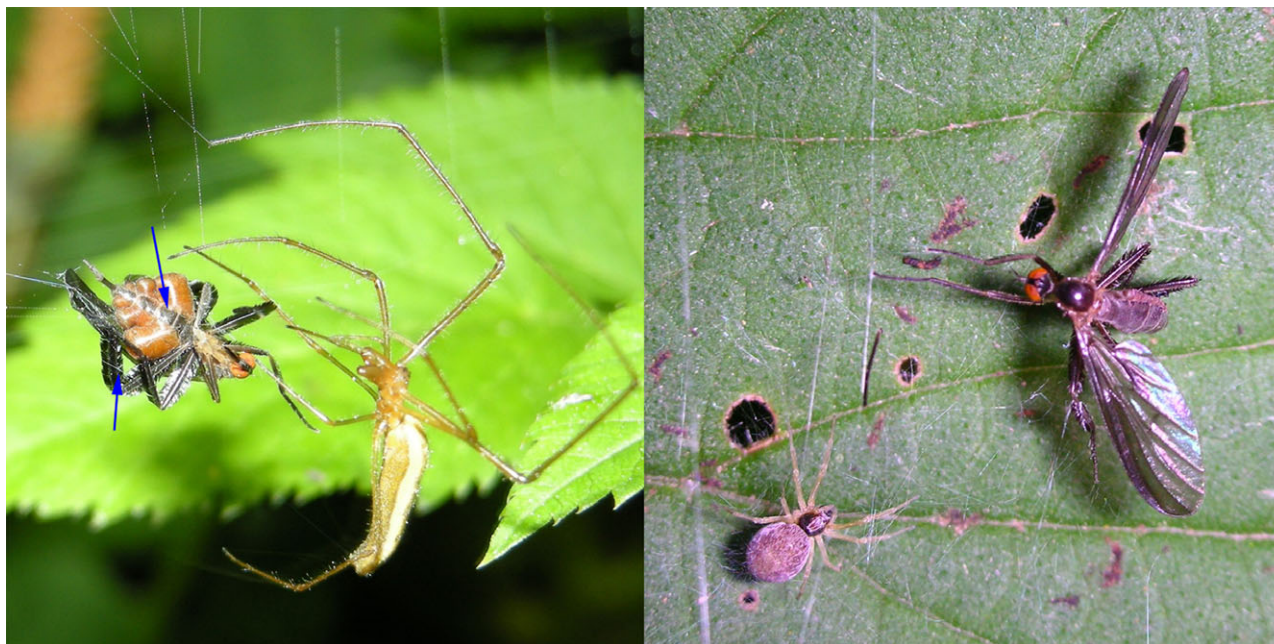
### THE SPECIES

*Rhamphomyia longicauda* swarms and mates during June at our study site on the banks of the Credit River, near Glen Williams (Halton Co., Ontario, Canada: 43°41'117"N, 79°55'3"W) (Gwynne & Bussière, 2002; Gwynne *et al.*, 2007; Bussière, Gwynne & Brooks, 2008; Wheeler *et al.*, 2012). By mid June at this site there are thousands of females displaying in several swarms for about an hour each dawn and dusk beneath gaps in the tree canopy. Although there is an even sex ratio of adults at the study site (Gwynne & Bussière, 2002), the typical swarm comprises about 90% females (Gwynne *et al.*, 2007). Each swarming female pulls her scale-covered legs (mainly the middle and hind legs) alongside a greatly inflated abdomen (Fig. 1), thus presenting a large silhouette to gift-bearing males approaching from below. Males ascend through the swarm and transfer a prey gift to a female and the copulating pair then joins a separate mating swarm (Newkirk, 1970; Funk & Tallamy, 2000). Unlike other empidines (Svensson & Petersson, 1987; Preston-Mafham, 1999; Daugeron & Grootaert, 2003; LeBas *et al.*, 2003), mating in this species occurs in flight. Females remain inflated while mating and eating the prey gift.

Our two spider predators have different web structures (Fig. 1). *Tetragnatha* (Tetragnathidae) preys on flying insects (Williams, Ambrose & Browning, 1995) including *R. longicauda* (Newkirk, 1970). *T. straminea* (about 1 cm long) builds a horizontal sticky orb web at dusk that is typically taken down each day. This spider is common near *R. longicauda* swarming sites so most prey females had been swarming and inflated when caught. The second spider, common in 2006 at our site (but not in 2007) is a *Dictyna* species (Dictynidae), < 5 mm in length, that constructs a more permanent non-sticky flat web on the upper surface of leaves. Web strands entangle the legs of flying insects including many Diptera (Judd, 1969; and X. Gwynne. unpublished). As *Dictyna* webs are present throughout the day, some of their *R. longicauda* female prey were uninflated individuals flying around prior to swarming.

### SAMPLING

We sampled prey and survivors for selection analysis throughout the 2006 and 2007 swarming seasons



**Figure 1.** The two spider predators: *Dictyna* with an uninflated female *R. longicauda* prey and (right) *Tetragnatha straminea* with a fully inflated female. The blue arrows point in left photograph point to the ornamental traits measured in this study. Top arrow: the third abdominal sternite, Bottom arrow: area of scales on the inside of the middle tibia.

(during June). We focussed on the areas around swarms studied by Wheeler *et al.* (2012) which included three swarms along an approximately 15 m section of north–south trail along a riverbank and a fourth swarm approximately 60 m further north on the trail. We collected all available prey from *Tetragnatha* and *Dictyna* spider webs within about 3 m of each swarm. *Tetragnatha* establish their webs at twilight just as *R. longicauda* started to swarm. In order to obtain intact prey we continuously scanned *Tetragnatha* webs and removed flies soon after they were captured. This was important as this species quickly wraps prey in silk. Webs of both spiders were positioned between about 0.5 m and 0.75 m above the ground. As flying *R. longicauda* are caught in webs, each time a female prey was collected, we used an insect net to sample flying female survivors from the swarm (flying females were between 0.5 and 1.5 m above ground) 1–2 m away from the prey location.

All flies were killed by freezing within an hour of being sampled before being preserved in 70% ETOH. As the abdomen ornament of the female began to deflate as soon as it was caught by a spider, we were unable to measure the area of the inflated abdomen as in Wheeler *et al.* (2012). Instead, the third abdominal sternite (plate), the best predictor of the size of the inflated abdomen (Wheeler, 2008) was used as a proxy of inflated abdomen size. *R. longicauda* prey of *Dictyna* spiders were collected from webs from late afternoon through to the end of the swarming period.

In contrast to the silk wrapping of prey prior to feeding by *Tetragnatha*, *Dictyna* spiders tend to quickly macerate their victims so that many parts of dance fly prey including the abdominal tergite were not intact and thus unavailable for measurement. However, legs were usually intact.

Traits were measured under a dissecting microscope fitted with a digital camera (LeicaDFC290) connected to a computer. We used the digital imaging programme ImageJ 1.45 software (<http://rsb.info.nih.gov/ij/download.html>). For *Tetragnatha* prey we measured thorax length, wing length, abdominal plate area, tibia length, and pinnate scale area (the area of scales on the inferior surface of the middle tibia). We measured both right and left structures for paired traits taking the mean, except when one of the body parts was damaged. In these cases only the undamaged structure was measured. Due to *Dictyna* damage to most prey, measurements were restricted to length and scale area of the middle leg tibia.

For 2006, we had a complete set of morphological measures for all three traits from 107 flies caught in *Tetragnatha* webs as well as 147 wild-caught flies (survivors). For 2007, we had measures for the same traits from 33 prey and 75 survivors. For analyses of viability selection exerted by both predators in 2006, we had a larger data set (because these analyses were only based on tibia length and tibial scale area) derived from 118 flies from *Tetragnatha* webs, 39



flies from *Dictyna* and 149 survivors. Numbers of survivors were typically greater than prey because more of the prey specimens were damaged.

#### MEASURING AND INTERPRETING SELECTION GRADIENTS

Three female morphological traits were included in the phenotypic selection analyses of viability imposed by *Tetragnatha* webs: length of the middle tibia (TL) (a proxy for overall size;  $r > 0.69$  for all pair-wise correlations with thorax length and wing length), scale area (SA), and abdominal plate area (AP) (trait descriptive statistics are summarized in Table 1). SA and AP were square root transformed prior to analyses since these measures of area (and their variances) are expected to scale linearly with TL (see Lande 1977). To facilitate analyses of selection on scale area independent of its correlate, tibia length, we used the residuals of (square root) TL after linear regression of SA on TL and used these values (rSA) in all analyses. Subsequently TL, rSA and AP were standardized within-year (mean = 0, standard deviation = 1).

Survivors were assigned an absolute fitness of 1 and prey an absolute fitness of 0. Relative fitness was defined as absolute survival divided by mean absolute fitness (Crow, 1958), calculated within-year. For each year separately, standardized linear selection gradients were estimated from a multiple linear regression of relative fitness on the three traits; standardized quadratic and correlational selection

gradients were estimated from a separate regression including the quadratic and cross-product terms (Lande & Arnold, 1983). Quadratic terms from the latter were doubled to obtain the correct univariate nonlinear gradients (Stinchcombe *et al.*, 2008). Our sampling design (i.e. roughly equal numbers of survivors and prey) was chosen to avoid the potential statistical problems associated with underrepresentation of the rarer class. However, this also distorts the quantitative estimates of selection gradients because this gives an inaccurate estimate of  $I$ , the opportunity for (or upper limit to) selection and, ideally, fitness data should be sampled in proportion to the actual frequency of their occurrence in the population (Lande & Arnold, 1983; Arnold & Wade, 1984). Although a method exists to correct the gradients for sampling bias (see Blanckenhorn *et al.* 1999), this requires additional estimates of the actual proportion of individuals in the population that are predated which were unavailable given the very large size of populations of both the fly and the spiders along the riverbanks. Thus, one must be cautious in interpreting quantitative differences in estimates between sampling dates as these may reflect true temporal variation in  $I$  (see Punzalan & Rowe, 2013) but also different degrees of sampling bias. Nonetheless, our emphasis is on the temporal consistency of the mode/direction of selection, for which interpretation is not qualitatively affected.

Significance testing of the gradients was conducted using multiple logistic regression (Janzen & Stern, 1998). Instances (i.e. among traits and samples) of statistically significant selection were visualized using thin plate splines generated using the 'Tps' function in package 'fields' in R (version 2.13.0). The detection of phenotypic selection is sometimes aided by estimating selection along the canonical axes of nonlinear selection (Phillips & Arnold, 1989; Blows & Brooks, 2003; Reynolds, Childers & Pajewski, 2010). We performed these analyses, though in the present study, analyses of selection along the canonical axes did not qualitatively alter our conclusions because the major axes of nonlinear selection were closely aligned with the original trait axes. Thus, selection gradients along canonical axes were amenable to biological interpretation (Conner, 2007). Nonetheless, we report the values of  $\theta$  and  $\lambda$ , which correspond to the linear and nonlinear selection gradients, respectively, when estimating selection along the canonical axes.

As is customary, the sign and magnitude of the estimated selection gradients (whether performed on original traits or canonical axes) were interpreted as estimates of the strength and form of selection (Lande & Arnold, 1983). Positive/negative values of the linear gradients ( $\beta$  and  $\theta$ ) correspond to selection favouring increased/decreased population mean

**Table 1.** Descriptive statistics for *Rhamphomyia longicauda* traits used in analyses of viability selection by *Tetragnatha straminea* in 2006 ( $N = 254$ ) and 2007 ( $N = 108$ )

	Tibia length	Scale area	Plate area	Means
2006				
Tibia length	0.036	<i>0.890</i>	<i>0.610</i>	2.781
Scale area	0.012	0.005	<i>0.584</i>	0.930
Plate area	0.006	0.002	0.003	0.579
2007				
Tibia length	0.041	<i>0.927</i>	<i>0.473</i>	2.855
Scale area	0.014	0.006	<i>0.425</i>	0.947
Plate area	0.006	0.002	0.003	0.557

Measured traits include tibia length, square root SA and square root abdominal plate area, prior to standardization. Residual SA was used for phenotypic selection analyses. Phenotypic variances (diagonal), covariances (lower) and correlations (upper/italics) for the three measured traits. Units for all measures are in mm.

values, roughly approximating directional selection (Lande & Arnold, 1983; Mitchell-Olds and Shaw, 1987). The nonlinear selection gradients ( $\gamma$  and  $\lambda$ ) correspond roughly to stabilizing, disruptive and correlational selection on traits/trait combinations. However, as these definitions are not strictly synonymous (see Mitchell-Olds and Shaw, 1987), we often refer to these terms as simply convex and concave selection, corresponding to decelerating (negative gradients) and accelerating (positive gradients) functions, respectively.

We used two complementary approaches to pairwise comparisons of multivariate selection (i.e. comparing viability selection on females in 2006 and 2007). First, we used a geometric approach to summarize the degree of concordance between patterns of selection observed in each sample. To compare patterns of linear selection between samples, we normalized the (column) vectors  $\beta$  associated with each sample (denoted by subscripts  $a$  and  $b$ ) and calculated the vector correlation:

$$\rho = \beta_a^T \beta_b,$$

whereby  $T$  indicates transpose (see Punzalan, Rodd & Rowe, 2010 for a similar approach). We used the same approach to quantify similarity in patterns of nonlinear selection by first diagonalizing each gamma matrix, and then measuring the vector correlation between the leading eigenvectors (i.e.  $\gamma_{\max}$ ) of each. The vector correlation is bounded between 1 and  $-1$ , with values close to the former (latter) indicating strong positive (negative) concordance and values closer to zero indicating weak concordance between patterns of selection.

In the second approach used to quantify similarity in patterns of selection, we used a sequential model building approach and partial  $F$ -tests (Bowerman & O'Connell, 1990). Briefly, this approach compares the fit between a model (predicting fitness as a function of trait values and year) that includes year\*trait interactions vs. the same model with these terms excluded; partial  $F$ -tests are used to test for significant differences in model fit. Following Chenoweth & Blows (2005), model comparisons and corresponding partial  $F$ -tests were conducted separately for linear ( $\beta$ ), quadratic ( $\gamma_{\text{II}}$ ) and correlational ( $\gamma_{\text{IJ}}$ ) selection gradients.

For *R. longicauda* caught by *Dictyna*, only the legs could be measured. Thus, for the 2006 analyses that compared *Tetragnatha* predation to *Dictyna*, we examined only a subset of traits (TL and rSA). We variance standardized both traits and relativized fitness according to the distributions pooled across individuals that succumbed to either predator (absolute fitness = 0) as well the survivors (absolute

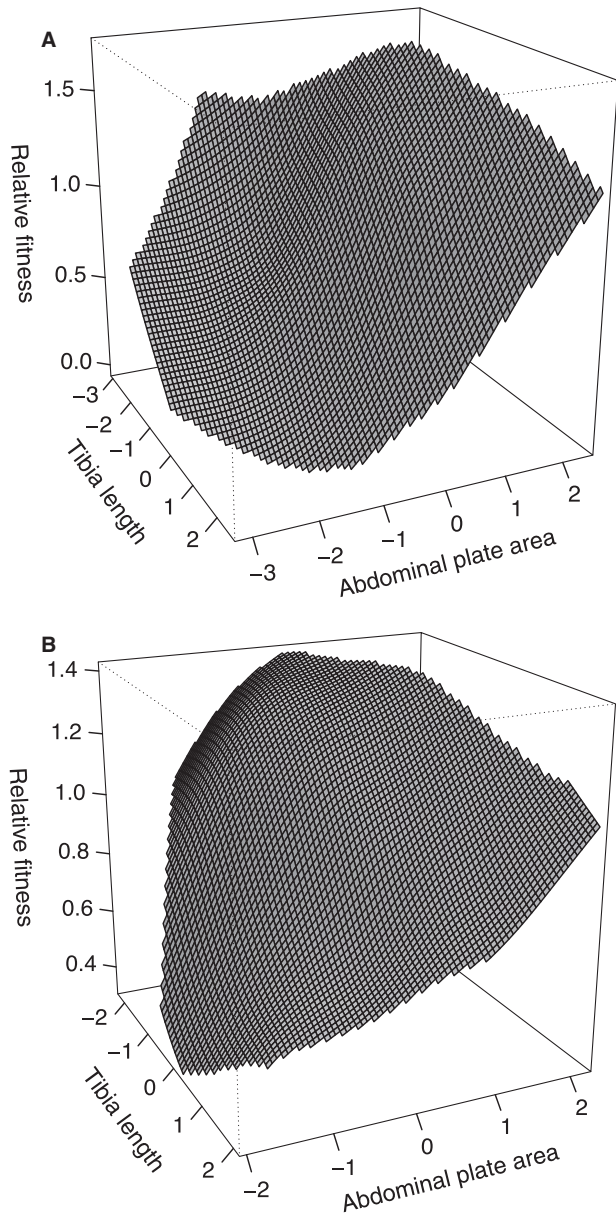
fitness = 1). Subsequently, we calculated selection gradients and statistical significance on these pooled data in the previously described manner. We should point out, however, that the nature of these data do not allow for a straightforward adding/partitioning of selection gradients (see Arnold & Wade, 1984; Wade & Kalisz, 1990; McGlothlin, 2011) because, here, fitness (survival) is mediated by the joint effects of selective agents (predators) acting simultaneously, rather than in independent episodes of selection (Hunt *et al.*, 2009). To examine possible differences between predators in their effects on the phenotypic distribution, we performed one-way ANOVAs, separately for the two traits, while classifying the data according to three categories (survivors, *Tetragnatha* prey, and *Dictyna* prey). This approach of comparing trait distributions of different samples of high and low fitness is roughly analogous to estimating selection differentials, whereby differences in the standardized distributions are compared before- and after-selection (Falconer, 1981; Endler, 1986; Brodie, Moore & Janzen, 1995). When appropriate, post-hoc tests were performed using Tukey's HSD.

## RESULTS

The overall pattern of linear viability selection on female secondary sexual traits due to predation by *T. straminea* spiders was consistent across the 2 years; the vector correlation between linear selection vectors was  $\rho = 0.94$ , with no evidence of significant year\*trait interactions using the partial  $F$ -tests (linear gradients:  $F_{3,354} = 1.741$ ,  $P = 0.158$ ). This reflects consistently significant positive linear selection on abdominal plate area but with negative linear selection on tibia length in both years (Fig. 2). Residual SA did not experience significant linear selection in either year (Table 2).

In terms of nonlinear viability selection, inspection of the elements of  $\gamma$  (Table 2) indicated significant concave selection on abdominal plate area in 2006 and a trend towards convex selection on residual SA in 2007. These between-year differences were supported by (1) a weak vector correlation ( $\rho = -0.16$ ) between the leading eigenvectors (M1 in 2006 and M3 in 2007), and (2) the partial  $F$ -tests that indicated most differences were with respect to the quadratic gradients ( $F_{3,348} = 3.43$ ,  $P = 0.018$ ) rather than differences in correlational gradients ( $F_{3,342} = 0.175$ ,  $P = 0.913$ ).

Selection analyses conducted on the major axes of nonlinear selection (i.e. after canonical rotation of gamma) were suggestive of linear selection acting along all three canonical axes in 2006 (Table 2), though most curvature was explained by axes M1



**Figure 2.** Thin-plate splines depicting relative fitness (survival) of *R. longicauda* as a function of tibia length and abdominal plate area due to viability selection imposed by *T. straminea* spiders in 2006 (A) and 2007 (B). Traits are variance standardized and both splines use a smoothing parameter of 0.5.

(83%) and M3 (16%). Patterns of selection generally recovered the one seen in original trait space, with selection favouring individuals with large values of abdominal plate area relative to the other traits (i.e. negative  $\theta$  on low M1 scores) and disfavouring individuals with high values of relative tibia length (M3 scores). Consistent with analyses on the original traits in 2007, we detected significant positive

( $\theta = 0.142$ ) but saturating selection ( $\lambda = -0.279$ ) on M3, an axis that distinguished residual SA from the other two traits and accounted for approximately 59% of the total curvature of the fitness surface (see Table 2 and Supporting Information, Fig. S1).

Phenotypic selection analyses of data that included both spider predators in 2006 (when *Dictyna* was abundant compared to other years) revealed significant positive linear selection on residual SA (i.e. favouring large SA) but no significant selection on tibia length. We did not detect significant nonlinear selection in original trait space, or after canonical analysis. However, again linear selection along the major axis of gamma supports the analysis performed on the original traits (Table 2). Recall that the other ornament trait, abdominal plate area could not be analysed as this trait was missing or damaged in most *Dictyna* prey.

Further analyses suggested that, although predation on *R. longicauda* females by *Dictyna* was lower (39 cases or 25% of 157 prey records) than by *Tetragnatha*, the former appears to have been the main agent of selection. That is, females with relatively small SAs succumbed to *Dictyna* predation compared to surviving *R. longicauda* while there was relatively little difference between female fly prey of *Tetragnatha* with respect to residual SA in these data ( $F_{2, 303} = 11.48$ ,  $P < 0.0001$ , Fig. 3). Average tibia length did not differ between predator categories, or between these categories compared to survivors ( $F_{2, 303} = 0.180$ ,  $P = 0.835$ , Fig. 3).

In summary, (1) viability selection due to mortality in sticky orb webs (*Tetragnatha*) over 2 years was relatively consistent, with linear selection favouring larger abdominal plates and shorter tibial lengths (with no significant selection on the second ornament, tibial SA); and (2), in the year when *Dictyna* spiders were abundant, viability selection that included both predators favoured larger scale ornaments with no significant selection on tibia length. Nonlinear selection was less consistent across studies, with some evidence of curvature of the fitness surface (albeit of different sign and on different traits) in both years.

## DISCUSSION

For sexually selected male traits, some studies have shown associations with increased mortality or risk (Kotiaho *et al.*, 1998; Quinn *et al.*, 2001; Robinson *et al.*, 2006; Hernandez-Jimenez & Rios-Cardenas, 2012; Ercit & Gwynne, 2015). Taken together, however, selection studies provide less support for opposing viability selection because meta-analyses show:



**Table 2.** Summarized estimates and corresponding standard errors (in parentheses) of phenotypic selection on *Rhamphomyia longicauda*, imposed by *Tetragnatha* (only) predation in 2006 and 2007 and by *Tetragnatha* and *Dictyna* (combined) in 2006

		$\beta$			$\gamma$						
		TL	rSA	AP	$\theta$	$\lambda$	TL	rSA	AP		
Viability selection imposed by <i>Tetragnatha</i>											
2006	TL	-0.292*** (0.063)	-0.036 (0.110)		M1	-0.467***	0.255*	0.205	0.086	-0.975	
	rSA	0.040 (0.050)	-0.001 (0.067)	-0.002 (0.068)	M2	-0.105*	-0.002	0.143	-0.988	-0.057	
	AP	0.421*** (0.063)	-0.061 (0.096)	-0.023 (0.070)	0.240* (0.110)	M3	-0.187***	-0.048	0.968	0.128	0.215
Linear model $R^2 = 0.161$ , $F_{3,250} = 15.98$ , $P < 0.0001$ ; Nonlinear model $R^2 = 0.184$ , $F_{9,244} = 6.11$ , $P < 0.0001$											
2007	TL	-0.239** (0.068)	0.032 (0.128)		M1	-0.248***	0.054	0.960	-0.237	-0.152	
	rSA	-0.081 (0.060)	-0.076 (0.066)	-0.260** (0.078)	M2	0.207	-0.137	0.155	-0.008	0.988	
	AP	0.246** (0.068)	-0.027 (0.103)	0.011 (0.07)	-0.132 (0.128)	M3	0.142*	-0.279*	-0.235	-0.972	0.029
Linear model $R^2 = 0.158$ , $F_{3,104} = 6.49$ , $P < 0.0001$ ; Nonlinear model $R^2 = 0.269$ , $F_{9,98} = 4.01$ , $P < 0.0001$											
Viability selection imposed by <i>Tetragnatha</i> and <i>Dictyna</i> combined in 2006											
	TL	-0.035 (0.058)	0.054 (0.082)		M1	0.066	0.057	-0.985	0.170		
	rSA	0.185** (0.058)	-0.022 (0.058)	-0.070 (0.078)	M2	0.176*	-0.073	0.170	0.985		
Linear model $R^2 = 0.033$ , $F_{2,303} = 5.23$ , $P = 0.006$ ; Nonlinear model $R^2 = 0.038$ , $F_{5,300} = 2.35$ , $P = 0.041$ .											

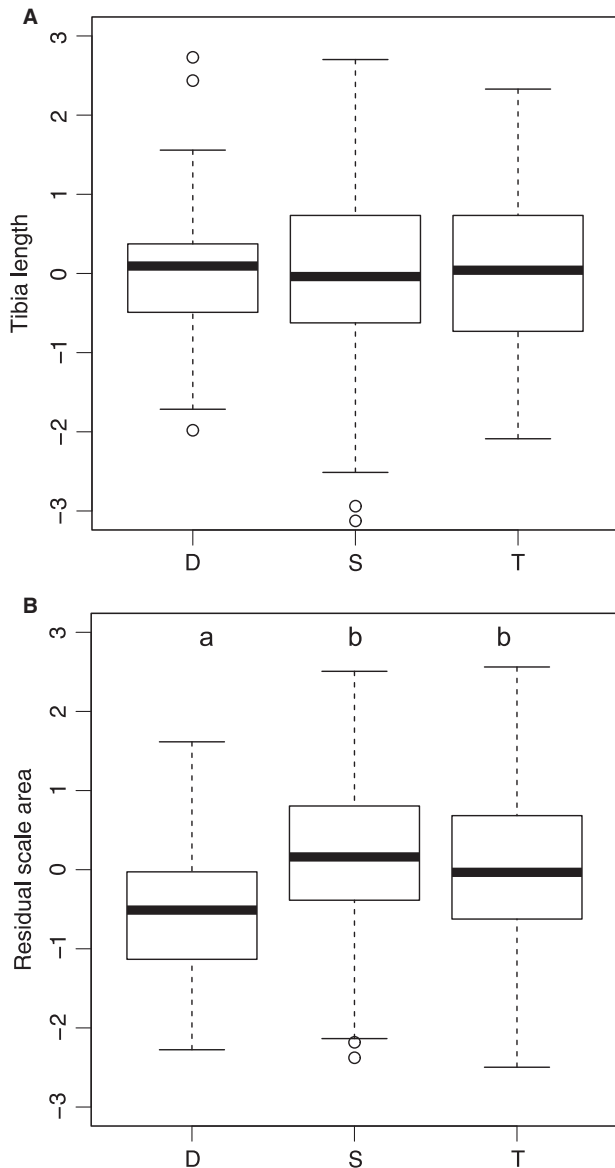
Reported are variance standardized selection gradients ( $\beta$  and  $\gamma$ ) as well as canonical selection gradients ( $\theta$  and  $\lambda$ ) for tibia length (TL), residual pinnate scale area (rSA) and abdominal plate area (AP). For the analyses of combined predation in 2006, estimates were restricted to TL and residual scale area (rSA). Asterisks indicate significance based on logistic regressions or permutation tests (see text) at 0.05 (\*), 0.01 (\*\*) and 0.001 (\*\*\*).

(1) significantly more cases in which the direction of selection on more than one fitness trait was the same than in which they were different (Kingsolver & Diamond, 2011); and (2) an overall positive relationship between sexually selected male traits and either adult male survivorship or male lifespan (Jennions, Moller & Petrie, 2001).

There have been no analyses of viability selection on sex-specific ornaments in females. These traits occur in the context of competition for access to males (Shuker, 2010) particularly when females compete for goods and services from males such as valuable mating gifts (Gwynne, 1991; Simmons & Parker, 1996). For *Rhamphomyia* dance flies using prey gifts there is sexual selection in the wild on

female ornamentation (LeBas *et al.*, 2003; and Wheeler *et al.*, 2012 on *R. longicauda*). The present study investigated viability selection in the wild on the two female ornaments of *R. longicauda*.

We studied the effects of spider predation on female traits. Although larger sample sizes would have improved our analyses (especially in the second season), these analyses showed consistent patterns of directional viability selection over two seasons (for dance flies killed by a spider that uses sticky orb webs: *T. straminea*: Fig. 1). However, viability selection from *T. straminea* contrasted for the traits. For one of the ornaments, abdominal plate size, consistent with some hypotheses and results on the evolution of male sexually selected traits (Wallace, 1889;



**Figure 3.** Boxplots illustrating differences in the trait distributions for tibia length (left panel) and residual (square root) SA (right panel) among sampled *R. longicauda*, belonging to three categories: survivors (S), *Dictyna* prey (D) and *Tetragnatha* prey (T). Both traits (y-axes) were variance standardized. Significant differences according to Tukey's HSD ( $\alpha = 0.05$ ) between groups are denoted by different lowercase letters.

Grafen, 1990; Jennions *et al.*, 2001), we found that viability selection in both years favoured larger abdominal plates in females. There was no significant viability selection on female tibial scale ornaments. However, for the length of the tibiae, viability selection from *T. straminea* predation was negative. This may indicate that females with long, scale-covered legs are less able to extricate themselves

from sticky orb webs that contact the whole body of the prey.

For the abdominal ornament, we note again that plate area was a proxy for a measure of the actual size of the large inflated abdomen displayed in swarms (Fig. 1). It is possible that the much smaller plate may be a poor trait to use in assessing the risk for a fully inflated female flying into a web. An inflated abdomen would likely contact a larger area of sticky web, possibly increasing predation risk. In fact, an experimental study showed a strong trend toward more inflated than uninflated female *R. longicauda* being entangled more often when released near webs (Gwynne *et al.*, 2007). Furthermore, abdominal plate area may represent the overall size (and vigour) of the female, i.e. our finding of positive viability selection on this trait may simply indicate larger individuals in higher condition are better able to escape from sticky webs.

Long-term viability selection for larger scale ornaments could lead to rapid elaboration of these ornaments given that sexual selection also tends to favour large scale ornament size in female *Rhamphomyia* (LeBas *et al.*, 2003; Wheeler *et al.*, 2012). We found a viability selective advantage for larger scale ornaments when the 2006 analysis included both *Tetragnatha* prey and prey from the nonsticky *Dictyna* webs that were abundant in that year. Evidence that viability selection in 2006 from *Dictyna* was more intense than from *Tetragnatha* is that the positive linear selection occurred despite *Dictyna* contributing only about 25% of the *R. longicauda* female kills sampled. However, the reduced number of traits in our analysis of fly kills from both spiders raises an alternative explanation for the difference between *Tetragnatha* results alone and the pooled ones with both predators: missing traits (including condition) in observational data subject to selection analysis can have strong effects on selection parameters (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987; Hadfield, 2008; Walker, 2014).

It remains possible, however, that differences between the effects of the two predators on female traits and viability may relate to how species differences in web structure interact with the traits. It may be easier for a flying *R. longicauda* female with large tibial scales, if in high condition, to pull away from a few non-sticky, leg-snaring web strands of *Dictyna* on a leaf surface than when her whole inflated body contacts a sticky orb (Fig. 1). Furthermore, more of the female prey captured by *Tetragnatha* than by *Dictyna* would have likely been inflated (Fig. 1) when caught not only because the temporary nocturnal webs of *Tetragnatha* are erected at about the same time as female swarming but also because many webs were close to swarming locations.



The effects of sexually selected traits on viability is a central one in understanding the evolution of sexual differences and is a question that can only be fully addressed using field studies. Many natural enemies are expected to contribute to mortality so that patterns of consistent selection as shown here, may change in seasons when certain predators become abundant. Differences in life history of each species and their natural enemies might explain some of the opposing patterns found for the effects of sexual traits on survival, e.g. the suggestion that sexually selected traits not costly to viability are those that advertise male quality (Jennions *et al.*, 2001) or that contrasting effects of sexually selected signalling on lifespan (e.g. in different species of grylline crickets: Hunt *et al.*, 2004; Judge *et al.*, 2008) might be explained by differences in length of the male breeding season (Judge *et al.*, 2008). However, our suggestion that differences in web structures may directly select for or against larger female traits raises the possibility that rather simple differences in the mechanisms used by important predators may also influence the patterns of viability selection on sexually selected traits. Insights into the ecological mechanisms of selection will be necessary to understand the temporal variation in the direction of selection often found in natural populations (Morrissey & Hadfield, 2012).

#### ACKNOWLEDGEMENTS

Thanks to Jill Wheeler, Kyla Ercit, Rosalind Murray and Luc Bussière for comments or discussion; to Kendra Lahut, and Vicki Simkovic for assistance in laboratory and field, and to four anonymous referees for constructive comments. The research was funded by an Natural Sciences and Engineering Council of Canada Discovery grant to DG and JH was funded by a University Royal Society Fellowship. Data are available on request to darryl.gwynne@utoronto.ca.

#### REFERENCES

- Andersson M. 1994.** *Sexual selection*. Princeton, NJ: Princeton University Press.
- Arnold SJ, Wade MJ. 1984.** On the measurement of natural and sexual selection: theory. *Evolution* **38**: 709–719.
- Blanckenhorn WU, Reuter M, Ward PI, Barbour AD. 1999.** Correcting for sampling bias in quantitative measures of selection when fitness is discrete. *Evolution* **53**: 286–291.
- Blows MW, Brooks R. 2003.** Measuring non-linear selection. *The American Naturalist* **162**: 815–820.
- Bowerman BL, O'Connell RT. 1990.** *Linear statistical models: an applied approach*. Belmont, CA: Duxbury Press.
- Brodie ED, Moore AJ, Janzen FJ. 1995.** Visualizing and quantifying natural selection. *Trends in Ecology & Evolution* **10**: 313–318.
- Bussière LF, Gwynne DT, Brooks R. 2008.** Contrasting sexual selection on males and females in a role-reversed swarming dance fly, *Rhamphomyia longicauda* Loew (Diptera: Empididae). *Journal of Evolutionary Biology* **21**: 1683–1691.
- Chenoweth SF, Blows MW. 2005.** Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *American Naturalist* **165**: 281–289.
- Chenoweth SF, Doughty P, Kokko H. 2006.** Can non-directional male mating preferences facilitate honest female ornamentation? *Ecology Letters* **9**: 179–184.
- Conner JK. 2007.** A tale of two methods: putting biology before statistics in the study of phenotypic evolution. *Journal of Evolutionary Biology* **20**: 17–19.
- Cronin H. 1991.** *The ant and the peacock: altruism and sexual selection from darwin to today*. Cambridge: Cambridge University Press.
- Crow JF. 1958.** Some possibilities for measuring selection intensities in man. *Human Biology* **30**: 1–13.
- Cumming JM. 1994.** Sexual selection and the evolution of dance fly mating systems (Diptera: Empididae; Empidinae). *Canadian Entomologist* **126**: 907–920.
- Darwin C. 1871.** *The descent of man and selection in relation to sex*. London: John Murray.
- Daugeron C, Grootaert P. 2003.** Assessment of monophyly of species-groups within Afrotropical Empidini (Diptera: Empididae: Empidinae), with a cladistic analysis of the *Empis setitarsus*-group. *Systematic Entomology* **28**: 339–360.
- Downes JA. 1970.** The feeding and mating behaviour of the specialized Empidinae (Diptera); observations on four species of *Rhamphomyia* in the high arctic and a general discussion. *Canadian Entomologist* **102**: 769–791.
- Endler JA. 1986.** *Natural selection in the wild*. Princeton, NJ: Princeton University Press.
- Ercit K, Gwynne DT. 2015.** Darwinian balancing selection: predation counters sexual selection in a wild insect. *Evolution* **69**: 419–430. doi:10.1111/evo.12579.
- Falconer DS. 1981.** *Introduction to quantitative genetics*. London, UK: Longman.
- Fisher RA. 1930.** *The genetical theory of natural selection*. Oxford: Clarendon Press.
- Funk DH, Tallamy DW. 2000.** Courtship role reversal and deceptive signals in the long-tailed dance fly, *Rhamphomyia longicauda*. *Animal Behaviour* **59**: 411–421.
- Grafen A. 1990.** Biological signals as handicaps. *Journal of Theoretical Biology* **144**: 517–546.
- Gwynne DT. 1991.** Sexual competition among females: what causes courtship-role reversal? *Trends in Ecology & Evolution* **6**: 118–121.
- Gwynne DT, Bussière LF. 2002.** Female mating swarms increase predation risk in a 'role-reversed' dance fly (Diptera: Empididae: *Rhamphomyia longicauda* Loew). *Behaviour* **139**: 1425–1430.
- Gwynne DT, Bussière LF, Ivy TM. 2007.** Female ornaments hinder escape from spider webs in a role-reversed swarming dance fly. *Animal Behaviour* **73**: 1077–1082.

- Hadfield JD. 2008.** Estimating evolutionary parameters when viability selection is operating. *Proceedings of the Royal Society B-Biological Sciences* **275**: 723–734.
- Hernandez-Jimenez A, Rios-Cardenas O. 2012.** Natural versus sexual selection: predation risk in relation to body size and sexual ornaments in the green swordtail. *Animal Behaviour* **84**: 1051–1059.
- Hunt J, Brooks R, Jennions MD, Smith MJ, Bentsen CL, Bussiere LF. 2004.** High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**: 1024–1027.
- Hunt J, Breuker CJ, Sadowski JA, Moore AJ. 2009.** Male-male competition, female mate choice and their interaction: determining total sexual selection. *Journal of Evolutionary Biology* **22**: 13–26.
- Janzen FJ, Stern HS. 1998.** Logistic regression for empirical studies of multivariate selection. *Evolution* **52**: 1564–1571.
- Jennions MD, Moller AP, Petrie M. 2001.** Sexually selected traits and adult survival: a meta-analysis. *Quarterly Review of Biology* **76**: 3–36.
- Judd WW. 1969.** Harvestmen and spiders and their prey on milkweed, *Asclepias syriaca* L., at London. *Ontario. Canadian Journal of Zoology* **47**: 159–161.
- Judge KA, Ting JJ, Gwynne DT. 2008.** Condition dependence of male life span and calling effort in a field cricket. *Evolution* **62**: 868–878.
- Kingsolver JG, Diamond SE. 2011.** Phenotypic selection in natural populations: what limits directional selection? *American Naturalist* **177**: 346–357.
- Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001.** The strength of phenotypic selection in natural populations. *American Naturalist* **157**: 245–261.
- Kotiaho J, Alatalo RV, Mappes J, Parri S, Rivero A. 1998.** Male mating success and risk of predation in a wolf spider: a balance between sexual and natural-selection. *Journal of Animal Ecology* **67**: 287–291.
- Lande R. 1977.** Statistical tests for natural selection on quantitative characters. *Evolution* **31**: 442–444.
- Lande R, Arnold SJ. 1983.** The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- LeBas NR, Hockham LR, Ritchie MG. 2003.** Nonlinear and correlational sexual selection on 'honest' female ornamentation. *Proceedings of the Royal Society of London Series (B)* **270**: 2159–2165.
- McGlothlin JW. 2011.** Combining selective episodes to estimate lifetime nonlinear selection. *Evolution* **65**: 1521–1522.
- Mitchell-Olds T, Shaw RG. 1987.** Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.
- Morrissey MB, Hadfield JD. 2012.** Directional selection in temporally replicated studies is remarkably consistent. *Evolution* **66**: 435–442.
- Newkirk MR. 1970.** Biology of the longtailed dance fly *Rhamphomyia longicauda* (Diptera: Empididae). *Annals of the Entomological Society of America* **63**: 1407–1412.
- Oliver JC, Robertson KA, Monteiro A. 2009.** Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proceedings of the Royal Society B-Biological Sciences* **276**: 2369–2375.
- Phillips PC, Arnold SJ. 1989.** Visualizing Multivariate Selection. *Evolution* **43**: 1209–1212.
- Preston-Mafham K. 1999.** Courtship and mating in *Empis (Xanthempis) trigramma* Meig., *E. tessellata* F. and *E. (Polyblepharis) opaca* F. (Diptera: Empididae) and the possible implications of 'cheating' behaviour. *Journal of Zoology* **247**: 239–246.
- Punzalan D, Rowe L. 2013.** Ecological correlates of mating success in a wild population of ambush bug. *Ecological Entomology* **38**: 429–432.
- Punzalan D, Rodd FH, Rowe L. 2010.** Temporal variation in patterns of multivariate sexual selection in a wild insect population. *American Naturalist* **175**: 401–414.
- Quinn TP, Hendry AP, Buck GB. 2001.** Balancing natural and sexual selection in sockeye salmon: interactions between body size, reproductive opportunity and vulnerability to predation by bears. *Evolutionary Ecology Research* **3**: 917–937.
- Reynolds RJ, Childers DK, Pajewski NM. 2010.** The distribution and hypothesis testing of eigenvalues from the canonical analysis of the gamma matrix of quadratic and correlational selection gradients. *Evolution* **64**: 1076–1085.
- Richards OW. 1927.** Sexual selection and allied problems in the insects. *Biological Reviews of the Cambridge Philosophical Society* **2**: 298–364.
- Robinson MR, Pilkington JG, Clutton-Brock TH, Pemberton JM, Kruuk LEB. 2006.** Live fast, die young: trade-offs between fitness components and sexually antagonistic selection on weaponry in Soay sheep. *Evolution* **60**: 2168–2181.
- Shuker DM. 2010.** Sexual selection: endless forms or tangled bank? *Animal Behaviour* **79**: E11–E17.
- Siepielski AM, DiBattista JD, Carlson SM. 2009.** It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters* **12**: 1261–1276.
- Siepielski AM, DiBattista JD, Evans JA, Carlson SM. 2011.** Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. *Proceedings of the Royal Society B-Biological Sciences* **278**: 1572–1580.
- Simmons LW, Parker GA. 1996.** Parental investment and the control of sexual selection: can sperm competition affect the direction of sexual competition. *Proceedings of the Royal Society of London B, Biological Sciences* **263**: 515–519.
- South SH, Arnqvist G. 2011.** Male, but not female, preference for an ornament expressed in both sexes of the polygynous mosquito *Sabethes cyaneus*. *Animal Behaviour* **81**: 645–651.
- Stinchcombe JR, Agrawal AF, Hohenlohe P, Arnold SJ, Blows MW. 2008.** Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* **62**: 2435–2440.
- Svensson EI, Gosden TP. 2007.** Contemporary evolution of secondary sexual traits in the wild. *Functional Ecology* **21**: 422–433.

- Svensson BG, Petersson E. 1987.** Sex-role reversed courtship behaviour, sexual dimorphism and nuptial gifts in the dance fly, *Empis borealis* (L.). *Ann. Zool. Fennici* **24**: 323–334.
- Svensson BG, Petersson E. 1988.** Non-random mating in the dance fly *Empis borealis*: the importance of male choice. *Ethology* **79**: 307–316.
- Svensson BG, Petersson E, Forsgren E. 1989.** Why do males of the dance fly *Empis borealis* refuse to mate? The importance of female age and size. *Journal of Insect Behavior* **2**: 387–395.
- Tigeros N, Mowery MA, Lewis SM. 2014.** Male mate choice favors more colorful females in the gift-giving cabbage butterfly. *Behavioral Ecology and Sociobiology* **68**: 1539–1547.
- Tobias JA, Montgomerie R, Lyon BE. 2012.** The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B-Biological Sciences* **367**: 2274–2293.
- Wade MJ, Kalisz S. 1990.** The causes of natural selection. *Evolution* **44**: 1947–1955.
- Walker JA. 2014.** The effect of unmeasured confounders on the ability to estimate a true performance or selection gradient (and other partial regression coefficients). *Evolution* **68**: 2128–2136.
- Wallace A. 1889.** *Darwinism: an exposition of natural selection with some of its applications*. McMillan and Co: London and New York.
- Wheeler J. 2008.** Sexual selection on female ornamentation in a role-reversed dance fly. Unpublished M.Sc., University of Toronto.
- Wheeler J, Gwynne DT, Bussière LF. 2012.** Stabilizing sexual selection for female ornaments in a dance fly. *Journal of Evolutionary Biology* **25**: 1233–1242.
- Williams DD, Ambrose LG, Browning LN. 1995.** Trophic dynamics of two sympatric species of riparian spider (Araneae: Tetragnathidae). *Canadian Journal of Zoology* **73**: 1545–1553.

#### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Cubic spline representations of selection on the major axes of nonlinear (viability) selection imposed by *Tetragnatha straminea* on *Rhamphomyia longicauda* in 2006 (Panel A) and 2007 (Panel B). Note that vectors M1 and M3 represent different combinations in each year.