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Effects of diapause and host species on some morphometric characters in *Trichogramma* (Hym.: Trichogrammatidae)

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Abstract. Adult morphology of *Trichogramma* is modified by environmental conditions during the preimaginal development. For instance, the low temperatures inducing diapause cause a decrease of the ratio 'length of longest seta on male flagellum/width of flagellum' in *T. evanescens.* The species of the host also influences numerous body ratios. So, in females, the development of tibiae with regard to wings is more important with *Scotia ipsilon* than with *Galleria mellonella.* These results confirm the necessity of performing comparative morphological studies on individuals reared in the same conditions.
Key words. Morphometry; diapause; host species; egg parasitoids; *Trichogramma brassicae*; *T. daumalae*; *T. evanescens.*

Trichogramma are minute Hymenoptera, parasitoids of insect eggs and especially of Lepidoptera eggs. The genus occurs worldwide and includes numerous species. Some species are used in biological control against agricultural pests.

On 2 March 1990, we collected two groups of Noctuid (Lepidoptera) eggs (two eggs each) on a poplar branch in Vaulx-en-Velin (Rhône, France). After their diapause, three *Trichogramma* females emerged from these eggs in the laboratory. Supplied with *Ephestia kuehniella* Zeller (Lep.: Pyralidae) eggs, they furnished males (by parthenogenesis, virgin diploid females furnish only haploid males) which permitted the species determination. Diagnostic characters, genitalia and esterases studied by electrophoresis (Est $1^{0.09}$, Est $2^{0.24}$, Est $4^{0.50bis}$, Est $4^{0.51bis}$, Est $5^{0.44}$), corresponded to those of *T. evanescens* Westwood. Est $2^{0.24}$ and Est $4^{0.51bis}$ alleles were not previously observed in the Vaulx-en-Velin population[1], but the former had been identified in the Rhône département[1] and the latter in other French populations[2]. However, these males from laboratory G1 generation showed unusually short antennal setae for the species: length of longest seta on flagellum/width of flagellum = 2.25 with n = 10 (range: 2.1 to 2.4), versus generally about 3.2. We suspected the diapause or the low temperatures the mothers underwent to be a possible cause of the unusual morphology. The aim of the study is to verify such a hypothesis, in this and other species of *Trichogramma.*

The host species can also obviously influence the *Trichogramma* morphology. Other experiments considered the effect of different host species on some wing and leg characters in females. Published data relating to similar characteristics in males were also examined.

Materials and methods

Materials. Effects of diapause on male antennal morphology were studied in two species: LB strain of *T. brassicae* Bezdenko and Mo strain of *T. evanescens* (table 1). Morphological characteristics were measured in three successive generations reared in *Ephestia kuehniella* eggs: the G0 generation submitted to diapause, the G1 and G2 generations not submitted to diapause. Experimental results obtained in the Mo strain were then compared to measurements obtained in the Vaulx-en-Velin population of *T. evanescens.*

Effects of host species on female wing and leg morphology were studied in three species: LB strain of *T. brassicae*, TV strain of *T. evanescens* and 82 strain of *T.*

Table 1. *Trichogramma* strains used in the study

Species	Strain	Origin	Host	
T. brassicae	LB*	Central Europe	*Ostrinia nubilalis*	(Pyralidae)
T. daumalae	115**	France (Haute-Garonne)	*Lobesia botrana*	(Tortricidae)
T. evanescens	Mo	France (Rhône)	*Noctua pronuba*	(Noctuidae)
	TV	France (Rhône)	?	(Noctuidae)
T. voegelei	82	France (Alpes-Maritimes)	*Pieris brassicae*	(Pieridae)

* strain used in biological control in France; ** strain studied by Dugast and Voegelé[3].

voegelei Pintureau (table 1). The strains were reared on *E. kuehniella* but characteristics were measured after one generation on *Galleria mellonella* L. (Lep.: Pyralidae) or *Scotia ipsilon* Hufn. (Lep.: Noctuidae). Published data[3] relating to similar characteristics in males concern the 115 strain of *T. daumalae* Dugast and Voegelé (table 1). In this species, characteristics were measured after rearing on *E. kuehniella* (host used for continuous rearing) or after one generation on *G. mellonella*, *Sitotroga cerealella* (Olivier) (Lep.: Gelechiidae) or *Philosamia cynthia* Drury (Lep.: Attacidae).

Methods. For the study of diapause effects, we used various rearing temperatures, but always 70 to 75% RH and 16L:8D (the photoperiod has virtually no effect on the entry into diapause in *Trichogramma*[4]): in G0, 24 °C for one day, 13 °C for 24 days, 2 °C for three months and 24 °C until the emergence in *T. evanescens*; 25 °C for one day, 13 °C for 20 days, 2 °C for 13 months and 25 °C until emergence in *T. brassicae*; in G1, 23 °C and 25 °C until emergence in *T. evanescens* and *T. brassicae*, respectively; in G2, between 20 and 23 °C until emergence in *T. evanescens*, 25 °C until emergence in *T. brassicae*. The width of male flagellum (width of fla.) and the length of longest seta on flagellum (L. seta on fla.) were measured on photos taken under a microscope. Insects were mounted under coverslips in Canada balsam. One-way variance analyses were carried out to compare each characteristic and their ratio in G0, G1 and G2 generations. The two species *T. evanescens* and *T. brassicae* were compared by *t*-tests. Correlations between measured variables were calculated.

For the study of host effects, the photoperiod and relative humidity were the same as in the preceding study, but temperature was constant at 23 °C for *T. brassicae*, *T. evanescens* and *T. voegelei*, or 25 °C for *T. daumalae*. In the first three species, four female characteristics were measured: forewing length, forewing width, length of mid femur, and length of mid tibia. Insects were mounted as in the preceding study, but measurements were made with a micrometer set up on a microscope. The effects of the two hosts on these characteristics were compared by *t*-tests. A discriminant factorial analysis was carried out on all data. The correlations and regressions between measured variables were calculated, notably to reveal isometric or allometric ratios. In *T. daumalae*, among the numerous male characters measured[3], we focused only on those homologous to the preceding ones: forewing length, forewing width, and length of hind tibia. After comparison by *t*-tests of the characteristics measured in insects reared on different hosts, distribution of means were graphically analyzed.

Results and discussion

Effects of diapause. There is a generation difference in the width of flagellum (G0 < G1 = G2) in *T. brassicae* but not *T. evanescens*, while differences are noted among generations in both species for the length of longest seta on flagellum (*T. brassicae*: G0 = G2 < G1; *T. evanescens*: G0 < G1 = G2). Only *T. evanescens* shows a difference (G0 < G1 = G2) in the ratio between the two characteristics (table 2). Assuming the noted

Table 2. Characteristic variation in males of *T. brassicae* (LB strain) and *T. evanescens* (Mo strain) evaluated by analysis of variance (F test), when individuals are submitted to different thermic treatments during development (G0 with diapause, G1 and G2 without diapause)

		Width of fla.		L. seta on fla.		L. seta on fla./Width of fla.	
		Mean ± SE	F test	Mean ± SE	F test	Mean ± SE	F test
T. brassicae	G0	2.40 ± 0.04[a] (1.96–2.86)	21.910	9.24 ± 0.14[a] (6.78–10.71)	14.253	3.88 ± 0.09 (2.92–4.83)	2.225
	G1	2.75 ± 0.04[b] (2.32–3.03)	(p = 0.0001)	10.28 ± 0.16[b] (6.43–11.42)	(p = 0.0001)	3.74 ± 0.06 (2.77–4.43)	(p = 0.114)
	G2	2.65 ± 0.04[b] (2.14–3.21)		9.66 ± 0.11[a] (8.21–10.71)		3.67 ± 0.07 (3.00–4.46)	
T. evanescens	G0	2.93 ± 0.05 (2.32–3.57)	0.853	8.15 ± 0.16[a] (5.36–9.28)	41.425	2.80 ± 0.06[a] (2.00–3.54)	23.122
	G1	2.89 ± 0.03 (2.32–3.21)	(p = 0.429)	9.46 ± 0.08[b] (8.57–10.71)	(p = 0.0001)	3.29 ± 0.04[b] (2.94–3.86)	(p = 0.0001)
	G2	2.97 ± 0.04 (2.32–3.39)		9.39 ± 0.08[b] (8.57–10.35)		3.19 ± 0.05[b] (2.78–4.15)	
Comparison between	G0	8.47**		5.02**		9.94**	
T. brassicae and	G1	2.78**		4.64**		6.43**	
T. evanescens	G2	5.62**		1.93		5.68**	

Means followed by different letters[(a, b)] differ significantly ($p < 0.05$) according to the Scheffe test. Range between minima and maxima is given in brackets below the mean. Comparison of *T. evanescens* and *T. brassicae* by the *t* test (** $p < 0.01$).
Width of fla.: width of flagellum of antenna; L. seta on fla.: length of longest seta on flagellum (in 1/100 mm). N = 32.

differences are due to diapause, i.e. ignoring possible effects of random variation or experimental conditions, this physiological state would act differently in the two species. It seems to decrease the width of fla. in *T. brassicae* (and perhaps to increase L. seta on fla. by maternal effect, i.e. by effect on the next generation G1), and to decrease L. seta on fla. and the ratio between the two characters in *T. evanescens*.

Logically, our *T. evanescens* individuals from the G1 of females diapausing in nature would have shown a ratio L. seta on fla./width of fla. between 2.94 and 3.86 (table 2). However, the values experimentally obtained are lower (2.1 to 2.4, as previously noted) and closer to G0 individuals diapausing in the laboratory than to G1. So, the Vaulx-en-Velin population and the Mo strain might differ genetically for these characteristics.

The results show that rearing temperature, with or without diapause, can influence male antennal characteristics to a greater or lesser extent according to *Trichogramma* species or populations. For instance, low

Table 3. Effects of host (*Galleria mellonella* and *Scotia ipsilon*) on the forewing length (Wl), the forewing width (Ww), the length of mid femur (Lf), and the length of mid tibia (Lt) in females of three *Trichogramma* species (*T. brassicae*, LB strain; *T. evanescens*, TV strain; *T. voegelei*, 82 strain)

Character	Species	Host		*t*-test
		G. mellonella	*S. ipsilon*	
Wl	*T. brassicae*	51.80 ± 0.37	56.70 ± 0.60	6.87 ($p < 0.001$)
	T. evanescens	50.43 ± 0.43	60.64 ± 0.40	17.21 ($p < 0.001$)
	T. voegelei	49.72 ± 0.36	55.18 ± 0.68	7.02 ($p < 0.001$)
Ww	*T. brassicae*	25.23 ± 0.28	28.20 ± 0.31	7.01 ($p < 0.001$)
	T. evanescens	25.01 ± 0.27	32.05 ± 0.30	16.93 ($p < 0.001$)
	T. voegelei	24.26 ± 0.37	27.55 ± 0.33	6.51 ($p < 0.001$)
Lf	*T. brassicae*	11.99 ± 0.06	12.57 ± 0.17	3.22 ($p = 0.002$)
	T. evanescens	11.41 ± 0.15	12.94 ± 0.15	7.11 ($p < 0.001$)
	T. voegelei	11.45 ± 0.12	12.49 ± 0.13	5.76 ($p < 0.001$)
Lt	*T. brassicae*	15.99 ± 0.08	17.30 ± 0.18	6.53 ($p < 0.001$)
	T. evanescens	15.03 ± 0.18	18.28 ± 0.14	14.10 ($p < 0.001$)
	T. voegelei	14.63 ± 0.17	16.63 ± 0.17	8.24 ($p < 0.001$)

Means ($\pm$SE) in 1/100 mm. N = 30.

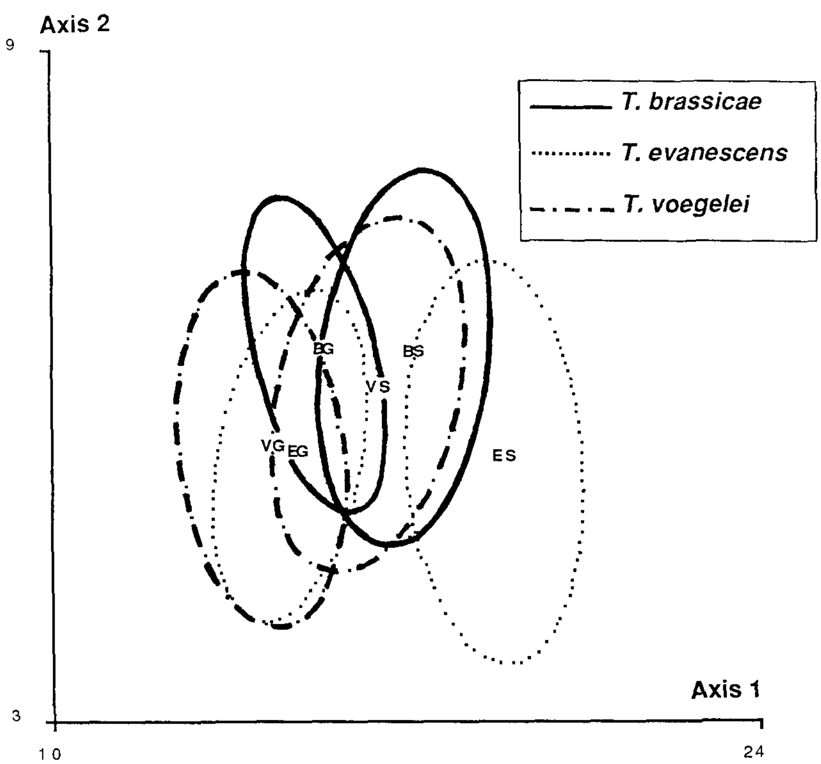

Figure 1. Discriminant factorial analysis carried out using four morphometric characteristics in females of *T. brassicae* (B, LB strain), *T. evanescens* (E, TV strain) and *T. voegelei* (V, 82 strain) reared on two hosts, *Galleria mellonella* (G) and *Scotia ipsilon* (S). Characteristics: forewing length, forewing width, length of mid femur, and length of mid tibia (N = 30). The ellipses enclose 95% of the observations.

temperatures seem directly to affect the width of fla. in *T. brassicae* and not in *T. evanescens*, and the L. seta on fla. in *T. evanescens* and not in *T. brassicae*. A temperature effect on morphometric parameters was already observed in other *Trichogramma* species[5], and authors emphasize the variability of characteristics such as L. seta on fla./width of fla. and advise caution when they are used in systematics.

Nevertheless, the two species studied are different for all characteristics and all generations, except for L. seta on fla. in G2 (table 2). We note that the mean of the ratio L. seta on fla./width of fla. is always higher in *T. brassicae* than in *T. evanescens*. Several values from the literature or calculated from literature data are available for *T. evanescens* (about 4, ref. 6; 2.6, ref. 7; 3.2, ref. 8, 9) and *T. brassicae* (at least 3.3, ref. 10; 3.5, refs. 7, 9). Whatever the thermic conditions, we always found mean values close to 3.1 in the first species and 3.8 in the second, which confirm the taxonomical value of this characteristic.

The two characteristics, width of fla. and L. seta on fla., are correlated (G0, G1 and G2 generations being grouped) in *T. brassicae* ($r = 0.401$, $n = 96$, $p < 0.01$) but not in *T. evanescens* ($r = 0.151$, $n = 96$, $p > 0.05$). This correlation seems very variable according to species ($r = 0.202$, $n = 100$, $0.05 > p > 0.01$ in a set of two species[7]; $r = -0.328$, $n = 90$, $p < 0.01$ in a set of three

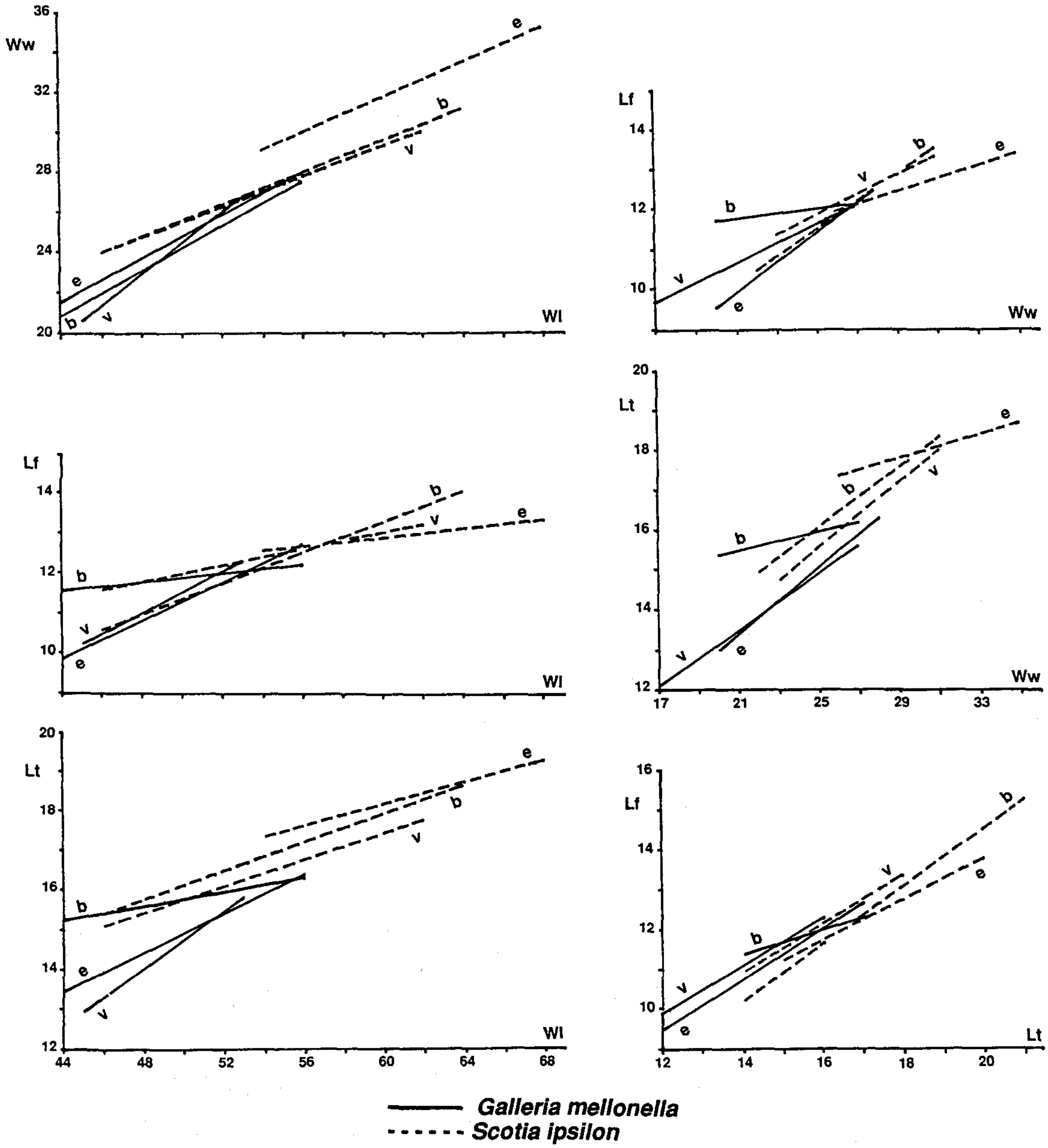

Figure 2. Regression lines for forewing length (Wl) versus forewing width (Ww), length of mid femur (Lf), or length of mid tibia (Lt), for Ww versus Lf or Lt, and for Lt versus Lf in females of *T. evanescens* (e, TV strain), *T. brassicae* (b, LB strain), and *T. voegelei* (v, 82 strain) reared on *Galleria mellonella* and *Scotia ipsilon*. N = 30. Measurements in 1/100 mm.

species[11]). As with other correlations, it might also vary with temperature[5].

Effect of hosts. In all the *Trichogramma* species studied, a host effect is observed on measured characteristics (table 3), *S. ipsilon* inducing greater values than *G. mellonella*. The discriminant factorial analysis (fig. 1) shows that females of the three closely related species are poorly distinguished when they are reared on the same host, which is also true of a wider range of characteristics[5]. With different hosts, we obtain a discrimination between individuals of the same species which is equal to or above the specific discrimination. So, the *T. evanescens* individuals reared on *S. ipsilon* are completely distinguishable from the *T. evanescens* reared on *G. mellonella*.

On *G. mellonella*, isometric ratios are often obtained between characteristics measured on different species. Exceptions are found in *T. brassicae* (Wl-Lf, Wl-Lt, Ww-Lf, Ww-Lt, Lt-Lf). On *S. ipsilon*, it is the same but exceptions are found in *T. evanescens* (Ww-Lf, Ww-Lt) (fig. 2). The allometric ratios between characters measured on different hosts are more frequent than those between characters measured on different species: Wl-Lf and Wl-Lt for all species, Ww-Lf and Ww-Lt for *T. brassicae* and *T. evanescens*, Wl-Ww for *T. voegelei*, and Lt-Lf for *T. brassicae*.

These results indicate an important host effect not only on the *Trichogramma* size, but also on the body ratios. A big host supports a bigger parasitoid than a small host. For instance, *S. ipsilon* produces *Trichogramma* with longer wings (mean: 55.18 to 60.64 according to the species) than *G. mellonella* (49.72 to 51.80). Ratios can also be strongly modified. For instance the mean ratio 'forewing width/length of mid tibia' reaches 1.66 and 1.75 in *T. evanescens* reared in *G. mellonella* and *S. ipsilon*, respectively, and 1.58 and 1.63 in *T. brassicae* reared in these two hosts.

The four measured characteristics are correlated (r included between 0.717 and 0.907, $n = 180$, $p < 0.01$) when all the values are grouped, whatever the *Tricho-*

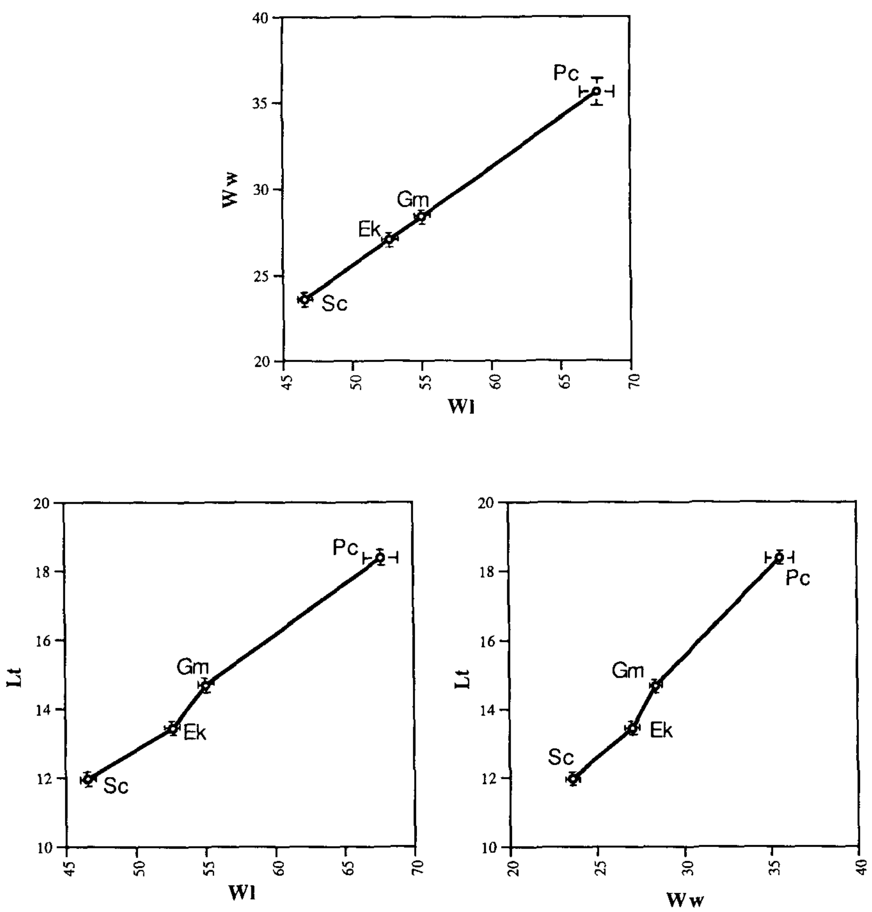

Figure 3. Graphic representation of the means (±SE) of three ratios, forewing length (Wl) to forewing width (Ww), Wl to length of hind tibia (Lt), and Ww to Lt, in males of *T. daumalae* (115 strain) reared on four hosts (Sc: *Sitotroga cerealella*; Ek: *Ephestia kuehniella*; Gm: *Galleria mellonella*; Pc: *Philosamia cynthia*). Means in 1/100 mm, N = 30. After data of Dugast and Voegelé[3]. The four means corresponding to one character are significantly different (*t*-test, $p < 0.001$; except for Ww on *G. mellonella* and *E. kuehniella*: $p < 0.01$). All correlations between mean characters on the different hosts are significant ($p < 0.01$): 1.000 for Wl-Ww, 0.997 for Wl-Lt and Ww-Lt.

gramma species (*T. evanescens*, *T. brassicae*, and *T. voegelei*) and the host (*G. mellonella* and *S. ipsilon*). Such a grouping does not allow a host effect on correlations to be revealed as for other characteristics[5].

In *T. daumalae*, the host effect is also obvious. All values increase from *S. cerealella* to *E. kuehniella*, *G. mellonella* and *P. cynthia*. The distribution of mean characteristics taken two by two (fig. 3) shows a very good linearity of hosts concerning the forewing (Wl and Ww) but a break between *E. kuehniella* and *G. mellonella* when the forewing is associated to the hind leg (Lt and Wl or Ww). This result shows that hosts with a size at least equal to that of *G. mellonella*, slightly above that of *E. kuehniella*, allow more development of the legs (at least of the tibae) compared to the wings. This phenomenon has already been observed in the three species *T. evanescens*, *T. brassicae* and *T. voegelei* when they were reared in *G. mellonella* and *S. ipsilon* (fig. 2). A more important development of the wing width with regard to the wing length was also noted in *T. evanescens*. Such modifications of body ratios by the species of the host confirm previous results[3,7,12–15].

Added to inter-population variability, the significant effects of host and temperature on imaginal morphology complicate *Trichogramma* systematics. This emphasises the need to carry out comparative studies on individuals reared in the same conditions, and justifies the recourse to characteristics not much influenced by environment, such as the electrophoretic analysis of enzymes[2,16–18].

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