



HOKKAIDO UNIVERSITY

Title	Growth rate adjustment of two <i>Drosophila</i> parasitoids in response to the developmental stage of hosts
Author(s)	Kohyama, Tetsuo I.; Onizawa, Kota; Kimura, Masahito T.
Citation	Ecological entomology, 42(6), 785-792 https://doi.org/10.1111/een.12444
Issue Date	2017-12
Doc URL	https://hdl.handle.net/2115/72068
Rights	This is the peer reviewed version of the following article: http://onlinelibrary.wiley.com/doi/10.1111/een.12444/abstract;jsessionid=8B1163E4F20D342CCD6A90D6B471B69D.f04t04 , which has been published in final form at 10.1111/een.12444. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Type	journal article
File Information	huscup_kohyama.pdf



Growth rate adjustment of two *Drosophila* parasitoids in response to the developmental stage of hosts

Authors

Tetsuo I. Kohyama¹, Kota Onizawa², Masahito T. Kimura³

Affiliations

¹Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido, 060-0810, Japan

²Graduate School of Environmental Science, Hokkaido University, Sapporo, Hokkaido, 060-0810, Japan

³University Museum, Hokkaido University, Sapporo, Hokkaido, 060-0810, Japan.

Running title

Growth rate adjustment of parasitoids

Correspondence

Tetsuo I. Kohyama

Tel: +81 11 706 2267

E-mail: tetsuo_kohyama@ees.hokudai.ac.jp

Abstract

1. Generalist koinobiont parasitoids often exhibit high flexibility in their development; their larvae shorten or prolong the developmental period depending on the host quality at parasitisation. However, flexibility of the growth rate of parasitoid larvae has rarely been investigated so far.

2. This study investigated how the koinobiont parasitoid wasps *Asobara japonica* and *Leptopilina ryukyuensis* regulate their larval growth when they parasitise host *Drosophila* larvae with varying larval periods.

3. In both parasitoid species, the preimaginal period was longer when they parasitised 1-day-old larvae of *Drosophila rufa* than when they parasitised older larvae of *D. rufa* or when they parasitised larvae of *Drosophila simulans*, a species with a shorter larval period than *D. rufa*. After host pupariation, *A. japonica* accelerated its growth, thereby showing a biphasic growth curve. On the other hand, *L. ryukyuensis* did not accelerate its growth after host pupariation.

4. Growth retardation of parasitoid larvae in 1-day-old *D. rufa* larvae would contribute to avoiding excess growth before host pupariation, because the excess growth of parasitoid larvae would have negative effects on host growth. The growth rate acceleration of *A. japonica* after host pupariation suggests that they enhance resource utilisation in a host that has reached maximum body mass. It remains uncertain as to why *L. ryukyuensis* does not show clear accelerated growth after host pupariation. Nonetheless, these results suggest that parasitoid larvae have an ability to detect the developmental stage of hosts in a species-specific manner.

Keywords: *Asobara japonica*; biphasic growth; koinobiont; larval-pupal parasitoid;
Leptopilina ryukyuensis; life-history strategy

Introduction

All insects show more or less non-genetic flexibility in morphological, physiological, developmental, and biochemical traits, some of which serve as adaptations to fluctuating environments (Bradshaw, 1965; West-Eberhard, 2003; Whitman & Agrawal, 2009). In this study, we focused on developmental flexibility in parasitoids. A parasitoid is an organism with a parasitic immature-stage that develops in or on the body of another organism, eventually killing it (Godfray, 1994). In generalist parasitoids that exploit a number of host species with differing growth periods or sizes, their larvae are expected to change their developmental schedule depending on the host species (Godfray, 1994; Harvey & Thompson, 1995). Even if they exploit a single host species, they must tune their development depending on the age or size of host individuals at parasitism (Mackauer & Sequeira, 1993; Harvey & Strand 2002; Harvey 2005).

How they adjust their developmental schedule is largely dependent on lifestyle, i.e. whether they have an idiobiont or koinobiont lifestyle (Askew & Shaw, 1986). Idiobiont larval parasitoids suppress host development at the time of parasitisation; therefore, their adult size is dependent on the age or size of the host at parasitisation. Koinobiont parasitoids, on the other hand, allow host eggs or larvae to develop after parasitisation. The advantage of the koinobiont lifestyle is obvious if they can oviposit in hosts in the early developmental stages. There are some advantages to oviposit in eggs or young larvae (Slansky, 1986). First, oviposition in eggs or young larvae is less risky for parasitoid females because younger targets are physically weaker. Second, it is easier to locate early instar larvae or nymphs because they are more numerous than late instars. In addition, attacking earlier-instar hosts is competitively advantageous, because they are less likely to be parasitized. However, earlier-instar hosts are resource-poor at least for idiobiont parasitoids that suppress host

development at parasitisation. In contrast, koinobiont parasitoids are able to exploit larger amounts of resources even if they oviposit in early-instar hosts, because hosts continue development after parasitisation. However, koinobiont parasitoid larvae must adjust their development rate if they are oviposited into hosts with differing growth periods or size. For example, the moth parasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae) and some others spent prolonged developmental periods and/or attained smaller terminal masses when it parasitized earlier-instar host larvae in comparison with later-instar larvae (Harvey *et al.*, 1994, 2000, 2012; Harvey & Strand 2002; Malcicka & Harvey, 2014). However, little is known how parasitoids adjust their own growth rate to shorten or prolong their developmental period in response to host quality.

Here, we studied the developmental responses of two *Drosophila* parasitoids, *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) and *Leptopilina ryukyuensis* Novković & Kimura (Hymenoptera: Figitidae). *Asobara japonica* is a thelytokous species occurring throughout Japan, whereas *L. ryukyuensis* is an arrhenotokous species occurring from southern Japan to Indonesia (Mitsui *et al.*, 2007; Novković *et al.*, 2011). These parasitoids are generalists, parasitising a number of *Drosophila* species (Diptera: Drosophilidae) that differ in larval period and size (Ideo *et al.*, 2008; Novković *et al.*, 2012). They are solitary larval-pupal koinobiont parasitoids, i.e. they oviposit in host larvae and allow them to develop until pupariation (formation of puparium, i.e., sclerotized skin of the final larval instar, within which pupa is formed), and one progeny survives in a single host even if multiple parasitoid eggs are oviposited. Larvae of koinobiont parasitoids are expected to retard or suppress their own development when they are oviposited in young host larvae or host species with longer larval periods. If the parasitoid larva grows too fast, the host might be killed before it grows large enough to provide sufficient resources to ensure survival and development of the parasitoid larva. This study aimed to verify this expectation using these

two parasitoid species and two host species differing in larval periods.

Materials and Methods

Laboratory strains

We used two parasitoid species *A. japonica* and *L. ryukyuensis*, and two host species *D. simulans* Sturtevant and *D. rufa* Kikkawa & Peng. The laboratory strains of *A. japonica* originated from a few females collected in Tokyo (35.7°N, 139.8°E) in June 2010, that of *L. ryukyuensis* from a few females collected in Iriomote-jima (24.2°N, 123.8°E) in March 2006, that of *D. simulans* from a few females collected in Tokyo in June 2007, and that of *D. rufa* from a female collected in Chiba (35.6°N, 140.1°E) in 1978. The larval period (from egg to puparium formation) is shorter in *D. simulans* (mean $\pm$ SD = 4.91 ± 0.34 days) than in *D. rufa* (7.22 ± 0.54 days) when reared at 23°C without parasitism. These strains were usually maintained at LD 15:9 h and 23°C until the experiments (March, 2016).

Parasitoid mortality and developmental time

Host larvae were parasitised 1-day after host eggs had been laid and 1 or 2 day(s) before pupariation (i.e., 4- and 3-day-old *D. simulans* larvae and 5- and 4-day-old *D. rufa* larvae by *A. japonica* and *L. ryukyuensis*, respectively). The earlier parasitisation in *D. simulans* was due to the fact that this host pupariates earlier, and the earlier parasitisation by *L. ryukyuensis* is due to this parasitoid being less resistant to the anti-parasitoid behaviors of older host larvae (T. I. Kohyama, personal observation). Twenty to 30 host larvae were placed with two or three parasitoid females in Petri dishes (diameter 3 cm) with small amounts of food medium, and were observed using a stereoscopic microscope. To avoid superparasitism, host larvae were transferred to new vials immediately after completion of oviposition by parasitoid females. In

all, 100 parasitized host larvae were prepared for each combination. Parasitized larvae were reared under LD 15:9 h at 23°C, and the number of host pupae (puparia) formed and the number of flies and wasps that emerged were examined every day. We defined the parasitoid mortality as the proportion of the cases in which a wasp did not emerge (i.e. a fly emerged, or the larvae died and neither a fly nor a wasp emerged).

Parasitoid growth trajectories

To investigate the growth trajectories of parasitoids, we additionally prepared host larvae parasitized as described earlier and reared them on *Drosophila* medium until use. We measured the body length of parasitoid larvae 1–12 or 13 days after being oviposited; host larvae or puparia were dissected in *Drosophila* Ringer's solution (128 mM NaCl, 6 mM KCl, 2 mM CaCl₂), and the body length of parasitoid larvae was measured using ImageJ 1.49v (<http://imagej.nih.gov/ij/>, 1997-2016) after their digital images were taken using a microscope. In 94% of the cases, five parasitoid larvae were measured for each time point.

Statistical analysis

We assessed the effects of host species and host age on parasitoid mortality and developmental time using generalised linear models (GLMs) with R 3.3.2 (R Core Team, 2016). We assumed that the observed distribution of parasitoid mortality followed a binomial distribution, and that of the developmental time followed a normal distribution. The models included 'host species', 'host age', and the interaction 'host species × host age' as explanatory variables. The significance of the explanatory variables was tested using a likelihood ratio test.

To analyze growth patterns of parasitoids, we employed a logistic equation that is widely used for the growth model of individual size as well as population size. The simple

logistic model described the change in body length of a parasitoid, Y (mm), as a function of its age since parasitisation t (day) as

$$\frac{dY}{dt} = rY \left(1 - \frac{Y}{K}\right), \quad (1)$$

where r (day^{-1}) is the potential relative growth rate for $Y \sim 0$, and K (mm) is the upper limit size of Y . We can extend this simple logistic equation (eqn 1), taking into account the time dependencies of r and K (Shinozaki, 1953). We assumed that there can be switching in the parasitoid growth rate at host pupariation, and that growth parameters (r , K) are constant at each phase (i.e. pre- and post-pupariation). We examined all of four possible models: model A, r and K are constant irrespective of phase; model B, only r is phase-dependent; model C, only K is phase-dependent; and model D, both r and K are phase-dependent. We defined the phase-dependent growth parameters, $r(t)$ and $K(t)$, as functions of the probability of host pupariation at t , $p(t)$:

$$r(t) = r_1(1 - p(t)) + r_2p(t), \quad (2)$$

and

$$K(t) = K_1(1 - p(t)) + K_2p(t), \quad (3)$$

where (r_1, K_1) and (r_2, K_2) are growth parameters at the pre- and post-host-pupariation phases, respectively. We assumed that the host pupariation probability $p(t)$ followed a logistic function:

$$p(t) = 1/[1 + \exp(-a - bt)]. \quad (4)$$

We estimated coefficients of $p(t)$, i.e. a and b in eqn 4, using a GLM with a binomial error distribution and a logit-link function based on the development time data (Figure S1 in File S1). We estimated growth parameters, r_i and K_i ($i = 1, 2$), using the Markov chain Monte Carlo method with Stan 2.12.0 (Stan Development Team, 2016) (R and Stan scripts are provided in File S2). The mean body length of parasitoid larvae at age t , $\mu(t)$, was calculated by numerical integration of the ordinary differential equation of the logistic growth model (eqn 1). The average length of parasitoid eggs measured at the day of parasitism ($t = 0$) was employed as an initial body size Y_0 . We assumed that the observed distribution follows a lognormal distribution with a mean of $\ln \mu(t)$ and standard deviation of σ . We employed the weakly informative or uninformative priors for the parameters (r_i , K_i and σ). We obtained posterior distributions of the parameters from four independent Markov chains, each of which contained 5000 iterations, including the initial 2500 iterations as burn-in, with the thinning interval of 10 iterations. Convergence of the chains was confirmed with the Gelman and Rubin's diagnostics (Gelman & Rubin, 1992). We compared the above-mentioned four models A-D, using the widely applicable information criterion (WAIC) (Watanabe, 2010).

Results

Parasitoid mortality and developmental time

Mortality of *A. japonica* was significantly higher in *D. rufa* than in *D. simulans* (GLM, $\chi^2_1 = 46.19$, $P < 0.001$), whereas mortality of *L. ryukyuensis* did not differ between these two hosts (GLM, $\chi^2_1 = 0.08$, $P = 0.775$; Fig. 1), indicating that these two parasitoid species differed in virulence against these two *Drosophila* species. No or only a few flies emerged in all combinations of hosts and parasitoids, indicating that neither host species is immunologically resistant to the parasitoids used in the present study. In addition, mortality of both parasitoids

was significantly higher when they were oviposited in younger host larvae (*A. japonica*: GLM, $\chi^2_1 = 6.89$, $P = 0.009$; *L. ryukyuensis*: GLM, $\chi^2_1 = 10.17$, $P = 0.001$; Fig. 1). No significant effects of the interaction of host species $\times$ host age on mortality were detected in either parasitoid species (*A. japonica*: GLM, $\chi^2_1 = 1.22$, $P = 0.27$; *L. ryukyuensis*: GLM, $\chi^2_1 = 0.89$, $P = 0.346$).

The preimaginal (i.e., egg-to-adult) period was shorter in *A. japonica* than in *L. ryukyuensis* (Fig. 1). In both parasitoid species, the preimaginal period was longer when they parasitized 1-day-old *D. rufa* larvae than when they parasitized 4- or 5-day-old *D. rufa* larvae or when they parasitized 1-day-old and older *D. simulans* larvae (Fig. 1). All fixed effects included in the GLMs were identified as significant on the preimaginal period of both *A. japonica* (host species, $\chi^2_1 = 50.68$, $P < 0.001$; host age, $\chi^2_1 = 50.68$, $P < 0.001$; host species $\times$ host age, $\chi^2_1 = 15.65$, $P < 0.001$) and *L. ryukyuensis* (host species, $\chi^2_1 = 170.23$, $P < 0.001$; host age, $\chi^2_1 = 66.97$, $P < 0.001$; host species $\times$ host age, $\chi^2_1 = 11.62$, $P < 0.001$).

Parasitoid growth trajectories

In *A. japonica*, model D, where both r and K were phase-dependent, was selected as the best-fit model, irrespective of host species or host age (Table 1; Table S1 and Figure S2 in File S1); growth rate was markedly increased after host pupariation ($r_1 < r_2$, Table 1; Fig. 2). In *L. ryukyuensis*, model D was also selected when it parasitised 4-day-old *D. rufa* larvae and 1-day-old *D. simulans* larvae (Table 1), but the estimated growth rate was decreased after host pupariation ($r_1 > r_2$, Table 1). Meanwhile, model A or C was selected when *L. ryukyuensis* parasitised 1-day-old *D. rufa* larvae and 3-day-old *D. simulans* larvae; i.e. at least r was constant before and after host pupariation (Table 1). The final body size, K_2 , did not differ by the host age at parasitisation in either species (Table 1; Fig. 2).

Figure 3 shows the trajectories of the relative growth rate of parasitoids. In both

parasitoid species, the relative growth rate decreases to nearly zero before host pupariation when they parasitised 1-day-old *D. rufa* larvae (Fig. 3), suggesting that parasitoid larvae retarded their own development. In *A. japonica*, the relative growth rate increased drastically after host pupariation irrespective of host species and age at parasitisation. In *L. ryukyuensis*, on the other hand, the trajectory of the relative growth rate approached a simple logistic growth curve when the period from parasitisation to host pupariation was shortened.

Discussion

In agreement with our expectation, *A. japonica* and *L. ryukyuensis* exhibited flexibility of larval growth as an adaptation to differences in host age at parasitisation or interspecific differences in host larval growth rate. Larvae of these parasitoids retarded their own growth before host pupariation when oviposited in 1-day-old *D. rufa* larvae. As a result, they had longer preimaginal periods when oviposited in 1-day-old *D. rufa* larvae than when oviposited in 4- or 5-day-old *D. rufa* larvae. It has also been reported in some koinobiont parasitoids that parasitisation in younger host larvae prolonged parasitoid developmental time (Harvey *et al.*, 1994, 2000, 2012; Harvey & Strand 2002; Malcicka & Harvey, 2014), but so far it has not been clear when and how parasitoid developmental time was prolonged. In this study, it was revealed that prolongation of preimaginal periods was caused by developmental retardation a little before host pupariation. The retardation at this time was apparently advantageous, as an excessive development of the parasitoid larvae before host pupariation would have negative effects on host development and eventually on the fitness of the parasitoids themselves.

By contrast, these parasitoids did not show apparent growth retardation when they parasitized 1-day-old *D. simulans* larvae. This difference could be attributable to the difference in larval period between *D. rufa* and *D. simulans*. The maximum body lengths of *A.*

japonica and *L. ryukyuensis* larvae before host pupariation are approximately 1.2 and 0.9 mm, respectively. These sizes could be the upper limit that these parasitoid larvae can attain before host pupariation. Parasitoid larvae attained this limit approximately at host pupariation when oviposited in 1-day-old *D. simulans* larvae, whereas they attained it 2 or 3 days after host pupariation when oviposited in 1-day-old *D. rufa* larvae, in which the parasitoid larvae retarded their growth. As mentioned earlier, if parasitoid larvae grow beyond the upper limit before host pupariation, the host larvae may die before pupariation or stop their development, resulting in smaller pupa. Conversely, if parasitoid larvae do not reach the limit by host pupariation, they do not need to retard their own growth. Thus, growth retardation of parasitoids may be favored when they need host individuals to reach a particular stage for completion of their development. However, it is also possible that this retardation is simply because of resource limitation for larvae that parasitise younger small larvae. It is not known which scheme is plausible. A study on the nutritional conditions of the hemolymph of host larvae may provide some cues to solve this problem.

The growth rate acceleration of *A. japonica* after host pupariation suggests that they enhance resource utilisation in a host that has reached maximum body mass. Rapid growth after host pupariation is obviously advantageous, as increases in development time after host pupariation might increase the risk of being attacked by secondary parasitoids and other predators. In addition, parasitoids with an ability to accelerate growth after host pupariation could have a wide host range, because they can use host species with short larval periods in addition to those with long larval periods. In fact, *A. japonica* has a very wide host range (Ideo *et al.*, 2008; Nomano *et al.*, 2015). It is also interesting how parasitoid larvae recognize host conditions or size, and nutritional or hormonal conditions may be a cue.

In contrast to *A. japonica*, *L. ryukyuensis* did not show such a biphasic growth curve, i.e. the body size growth rate did not change significantly after host pupariation. The

preimaginal development time was about 5 days shorter in *A. japonica* than in *L. ryukyuensis* regardless of which host it parasitised. This could be partly due to *A. japonica* accelerating its development after host pupariation, whereas *L. ryukyuensis* did not. It may also be caused by the difference in relative size of egg mass to adult or pupal mass (Donnell & Hunter 2002). If we assume that egg mass is correlated with egg length, egg mass is larger in *A. japonica* than in *L. ryukyuensis*, because egg length was approximately 1.5 times larger in *A. japonica* (T. I. Kohyama, unpublished). On the other hand, the adult mass should not differ much between these two species, because both of them almost completely consume the host pupae. Thus, it is plausible that *A. japonica*, which has a larger relative egg size, has a shorter preimaginal period. However, Donnell & Hunter (2002) further suggested that the relative egg size does not strictly determine the preimaginal period. Further study is needed on factors affecting the preimaginal development.

The results of the present study indicated that these two parasitoid species have different development strategies: *A. japonica* has evolved larger eggs, a short preimaginal period, and a capacity to accelerate development after host pupariation, whereas *L. ryukyuensis* has evolved small eggs and a longer preimaginal period without growth rate acceleration. Which strategy is advantageous could be dependent on environmental conditions. Interspecific competition may be such a condition. The two study species are solitary parasitoids, i.e. even if more than one egg is oviposited in a host individual, only one parasitoid survives, as a result of competition. In this respect, it was indicated that the first larva to hatch excluded subsequent larvae through physical attack (Harvey *et al.*, 2013) and larger eggs hatch earlier than smaller eggs (Donnell & Hunter, 2002). Thus, species with larger eggs, such as *A. japonica*, may have an advantage in competition over those having smaller eggs, such as *L. ryukyuensis*. In fact, an *Asobara* species, *A. tabida*, was superior in competition with a *Leptopilina* species, *L. heterotoma* (Bonsall *et al.*, 2002; but see van

Stein-van Liemp, 1982). In addition, environmental conditions of host insects could affect the developmental strategy of parasitoids, i.e., parasitoids that attack hosts living in concealed habitats have longer preimaginal periods, whereas those attacking apparent hosts have shorter preimaginal periods (Harvey & Strand, 2002). For further understanding, it is necessary to investigate trade-offs associated with life-history traits, including developmental trajectories.

In conclusion, our results clearly suggested that the two *Drosophila* parasitoids, *A. japonica* and *L. ryukyuensis*, adjust their own growth rates in response to the developmental stage of hosts, i.e. they delayed growth when they parasitised host larvae that must grow for a relatively long period (i.e. >5 days at 23°C) until pupariation. This may be because these larval-pupal parasitoids will suffer low fitness if they adversely affect the larval growth of hosts. It was further observed that *A. japonica* switched growth phase at host pupariation and showed accelerated growth after host pupariation. Such biphasic growth has not been reported previously and provides novel insights into the developmental adaptation in parasitoids. For further understanding of development strategies of koinobiont parasitoids, it is important to investigate how parasitoid growth rate changes with the host developmental stage, because they exhibit wide variation in the host developmental stage they attack.

Acknowledgements

We thank M. Watada for providing *Drosophila* strains, and T. S. Kohyama for fruitful discussion. This study was partly supported by a Grant-in-Aid to MTK (No. 23370005) from the Japan Society for the Promotion of Science. TIK and MTK designed the project. TIK, KO and MTK collected data. TIK performed data analysis. TIK and MTK wrote the initial draft of the paper. The authors declare that they have no conflict of interests.

References

- Askew, R.R. & Shaw, M.R. (1986) Parasitoid communities: Their size, structure and development. In *Insect Parasitoids* (ed. by Waage, J.K. & Greathead, D.). Academic Press, London, pp. 225–264.
- Bonsall, M.B., Hassell, M.P. & Asefa, G. (2002) Ecological trade-offs, resource partitioning, and coexistence in a host-parasitoid assemblage. *Ecology*, **83**, 925–934.
- Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115–155.
- Donnell, D.M. & Hunter, M.S. (2002) Developmental rates of two congeneric parasitoids, *Encarsia formosa* and *E. pergandiella* (Hymenoptera: Aphelinidae), utilizing different egg provisioning strategies. *Journal of Insect Physiology*, **48**, 487–493.
- Gelman, A. & Rubin, D.B. (1992) Inference from iterative simulation using multiple sequences. *Statistical Science*, **7**, 457–472.
- Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, NJ, USA.
- Harvey, J.A. (2005) Factors affecting the evolution of development strategies in parasitoid wasps: The importance of functional constraints and incorporating complexity. *Entomologia Experimentalis et Applicata*, **117**, 1–13.
- Harvey, J.A., Gols, R., Vet, L.E.M. & Marjolein Kruidhof, H. (2012) Development of a hyperparasitoid wasp in different stages of its primary parasitoid and secondary herbivore hosts. *Journal of Insect Physiology*, **58**, 1463–1468.
- Harvey, J.A., Harvey, I.F. & Thompson, D.J. (1994) Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. *Ecology*, **75**, 1420–1428.
- Harvey, J.A., Kadash, K. & Strand, M.R. (2000) Differences in larval feeding behavior correlate with altered developmental strategies in two parasitic wasps: implications for

- the size-fitness hypothesis. *Oikos*, **88**, 621–629.
- Harvey, J.A., Poelman, E.H. & Tanaka, T. (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. *Annual Review of Entomology*, **58**, 333–351.
- Harvey, J.A. & Strand, M.R. (2002) The developmental strategies of endoparasitoid wasps vary with host feeding ecology. *Ecology*, **83**, 2439–2451.
- Harvey, J.A. & Thompson, D.J. (1995) Developmental interactions between the solitary endoparasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae), and two of its hosts, *Plodia interpunctella* and *Corcyra cephalonica* (Lepidoptera: Pyralidae). *European Journal of Entomology*, **92**, 427–435.
- Ideo, S., Watada, M., Mitsui, H. & Kimura, M.T. (2008) Host range of *Asobara japonica* (Hymenoptera: Braconidae), a larval parasitoid of drosophilid flies. *Entomological Science*, **11**, 1–6.
- Mackauer, M. & Sequeira, R. (1993) Patterns of development in insect parasites. In *Parasites and Pathogens of Insects, Volume 1: Parasites* (ed. by Beckage, N.E., Thompson, S.A. & Federici, B.A.). Academic Press, San Diego, CA, USA.
- Malcicka, M. & Harvey, J.A. (2014) Trade-offs between developmental parameters of two endoparasitoids developing in different instars of the same host species. *Biological Control*, **74**, 52–58.
- Mitsui, H., Achterberg, K. van, Nordlander, G. & Kimura, M.T. (2007) Geographical distributions and host associations of larval parasitoids of frugivorous Drosophilidae in Japan. *Journal of Natural History*, **41**, 1731–1738.
- Nomano, F.Y., Mitsui, H. & Kimura, M.T. (2015) Capacity of Japanese *Asobara* species (Hymenoptera; Braconidae) to parasitize a fruit pest *Drosophila suzukii* (Diptera; Drosophilidae). *Journal of Applied Entomology*, **139**, 105–113.
- Novković, B., Mitsui, H. Suwito, A. & Kimura, M.T. (2011) Taxonomy and phylogeny of

- Leptopilina* species (Hymenoptera: Cynipoidea: Figitidae) attacking frugivorous drosophilid flies in Japan, with description of three new species. *Entomological Science*, **14**, 333–346.
- Novković, B., Oikawa, A., Murata, Y., Mitsui, H. & Kimura, M.T. (2012) Abundance and host associations of parasitoids attacking frugivorous drosophilids on Iriomote-jima, a subtropical island of Japan. *European Journal of Entomology*, **109**, 517–526.
- R Core Team (2016) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. [WWW document].
<https://www.R-project.org/> [accessed on 20 February 2017].
- Shinozaki, K. (1953) On the generalization of the logistic curve. I. General discussions. *The Journal of the Osaka City Medical Center*, **2**, 143–147 (in Japanese).
- Slansky, F. (1986) Nutritional ecology of endoparasitic insects and their hosts: An overview. *Journal of Insect Physiology*, **32**, 255–261.
- Stan Development Team (2016) Stan Modeling Language Users Guide and Reference Manual, Version 2.12.0. [WWW document]. <http://mc-stan.org/> [accessed on 20 February 2017].
- van Strien-van Liempt, W.T.F.H. (1982) The competition between *Asobara tabida* Nees von Esenbeck, 1834) and *Leptopilina heterotoma* (Thomson, 1862) in multiparasitized hosts. *Netherlands Journal of Zoology*, **33**, 125–163.
- Watanabe, S. (2010) Asymptotic Equivalence of Bayes Cross Validation and Widely Applicable Information Criterion in Singular Learning Theory. *Journal of Machine Learning Research*, **11**, 3571–3594.
- West-Eberhard, M.J. (2003) *Developmental plasticity and evolution*. Oxford University Press, New York, NY, USA.
- Whitman, D.W. & Agrawal, A.A. (2009) What is phenotypic plasticity and why is it important? In *Phenotypic plasticity of insects* (ed. by Whitman, D.W. & Ananthakrishnan,

T.N.). Science Publishers, Enfield, NH, USA, pp. 1–63.

Figure legends

Figure 1. Mortality (a, b) and female developmental time (c, d) of the parasitoids *Asobara japonica* and *Leptopilina ryukyuensis* when they parasitized *Drosophila rufa* (Dr) and *D. simulans* (Ds). Numbers above bars and boxes indicate the number of individuals used. Colored bar segments in the stacked bar charts (a, b) indicate the proportions of the cases where parasitoids did not emerge: light grey, a fly emerged; dark grey, larvae died and neither a fly nor a wasp emerged. In the box plots (c, d) the bottom, middle, and top lines are 25th, 50th, and 75th quartiles by respectively; whiskers indicate 1.5 times the interquartile range; and black circles are outliers.

Figure 2. The body size trajectories of *Asobara japonica* (Aj; a-e) and *Leptopilina ryukyuensis* (Lr; e-h) when they were parasitized *Drosophila rufa* (Dr) and *D. simulans* (Ds). Each point represents body length (mm) of a single parasitoid. Circles, triangles, and squares indicate the stage of the parasitoid; embryos (circle), larvae (triangle), and pupae (square). Open and closed symbols represent the stage of host; larvae (open) and pupae (closed). The grey scale gradient bar indicates the probability of host pupariation, from light gray for 0 to dark grey for 1. Solid black curves correspond to the posterior median estimates of the best-fit growth model (see Table 1). Each thin gray curve corresponds to a posterior sample of parameters, providing the uncertainty of the predictions.

Figure 3. Predicted relative growth rates of *Asobara japonica* (Aj; a-e) and *Leptopilina ryukyuensis* (Lr; e-h) when they parasitized *Drosophila rufa* (Dr) and *D. simulans* (Ds). The grey scale gradient bar indicates the probability of host pupariation, from light gray for 0 to dark grey for 1. Solid black curves correspond to the posterior median estimates of the best-fit

growth model (see Table 1). Each thin gray curve corresponds to a posterior sample of parameters, providing the uncertainty of the predictions.

Supporting Information

Additional file S1:

Table S1. The widely applicable information criterion values calculated for each model.

Figure S1. Estimated temporal changes of host pupariation probability after parasitisation.

Figure S2. The median growth curves predicted by four growth models.

Additional file S2:

R and Stan codes used for the Bayesian estimation of parameters of the growth models.

Tables

Table 1 Medians of posterior distributions of growth parameters. Lower and upper 95% highest posterior density intervals are given in parentheses.

Parasitoid species	N	Best-fit	Growth rate, r		Upper limit size, K	
Host species (age)		model*	r_1	r_2	K_1	K_2
<i>A. japonica</i>						
<i>D. rufa</i> (1 day)	57	D	0.98 (0.44, 1.94)	6.37 (3.69, 10.62)	0.76 (0.67, 0.86)	2.26 (2.08, 2.41)
<i>D. rufa</i> (5 days)	65	D	0.49 (0.40, 0.68)	4.31 (2.12, 10.49)	1.57 (1.08, 2.22)	2.26 (2.20, 2.31)
<i>D. simulans</i> (1 day)	65	D	0.51 (0.30, 0.81)	1.70 (1.22, 2.37)	1.06 (0.74, 1.83)	2.17 (2.08, 2.30)
<i>D. simulans</i> (4 days)	65	D	0.54 (0.36, 0.91)	1.18 (0.95, 1.46)	1.19 (0.70, 2.07)	2.25 (2.17, 2.35)
<i>L. ryukyuensis</i>						
<i>D. rufa</i> (1 day)	65	C	0.64 (0.53, 0.75)		0.80 (0.72, 0.89)	1.82 (1.67, 1.97)
<i>D. rufa</i> (4 days)	70	D	0.83 (0.55, 1.19)	0.30 (0.09, 0.56)	0.80 (0.56, 1.30)	1.80 (1.49, 2.56)
<i>D. simulans</i> (1 day)	70	D	0.92 (0.65, 1.21)	0.35 (0.27, 0.44)	0.84 (0.57, 1.32)	2.04 (1.88, 2.24)
<i>D. simulans</i> (3 days)	70	A	0.49 (0.46, 0.54)		2.09 (1.96, 2.24)	

*The best-fit growth model was selected based on the widely applicable information criterion.

Models A, simple logistic model, i.e. r and K are constant irrespective to the host stage; model B only r is phase-dependent; model C, only K is phase-dependent; model D, both r and K are phase-dependent.

Figures

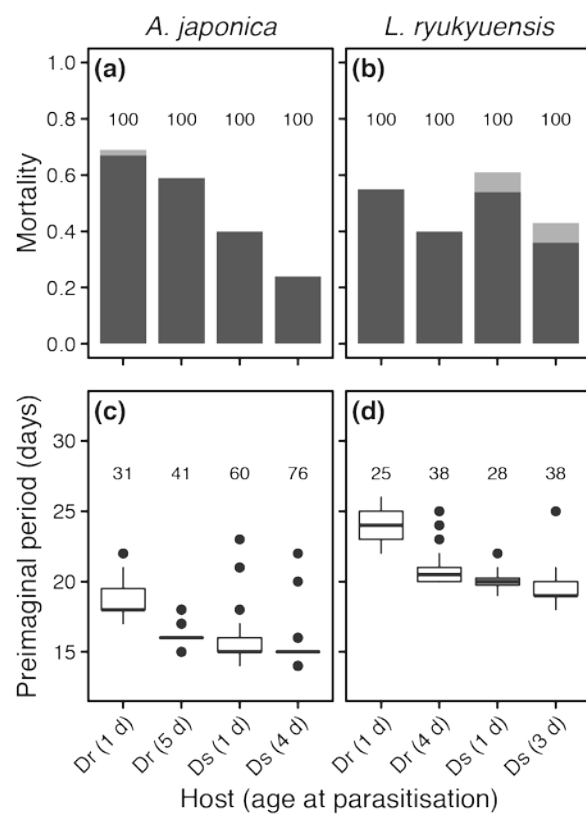


Figure 1

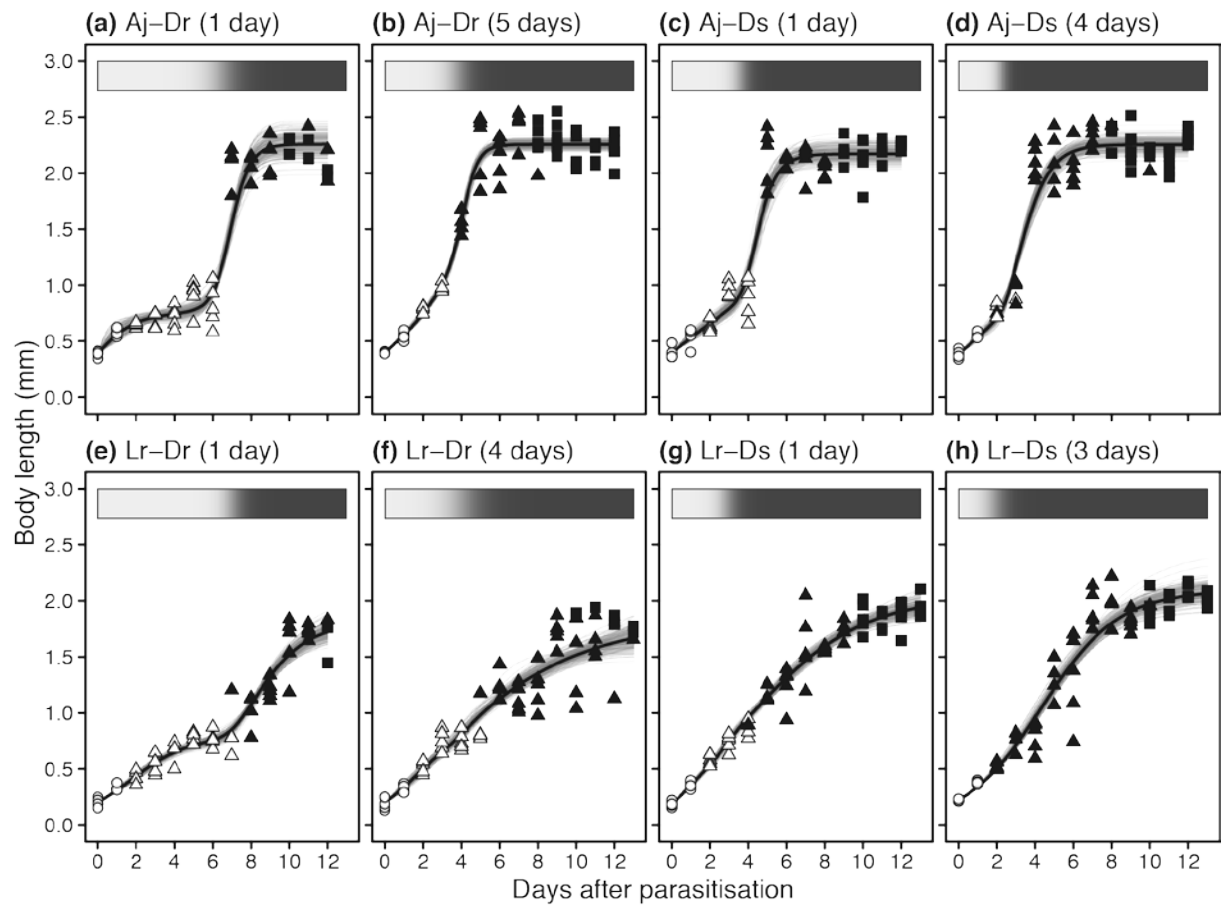


Figure 2

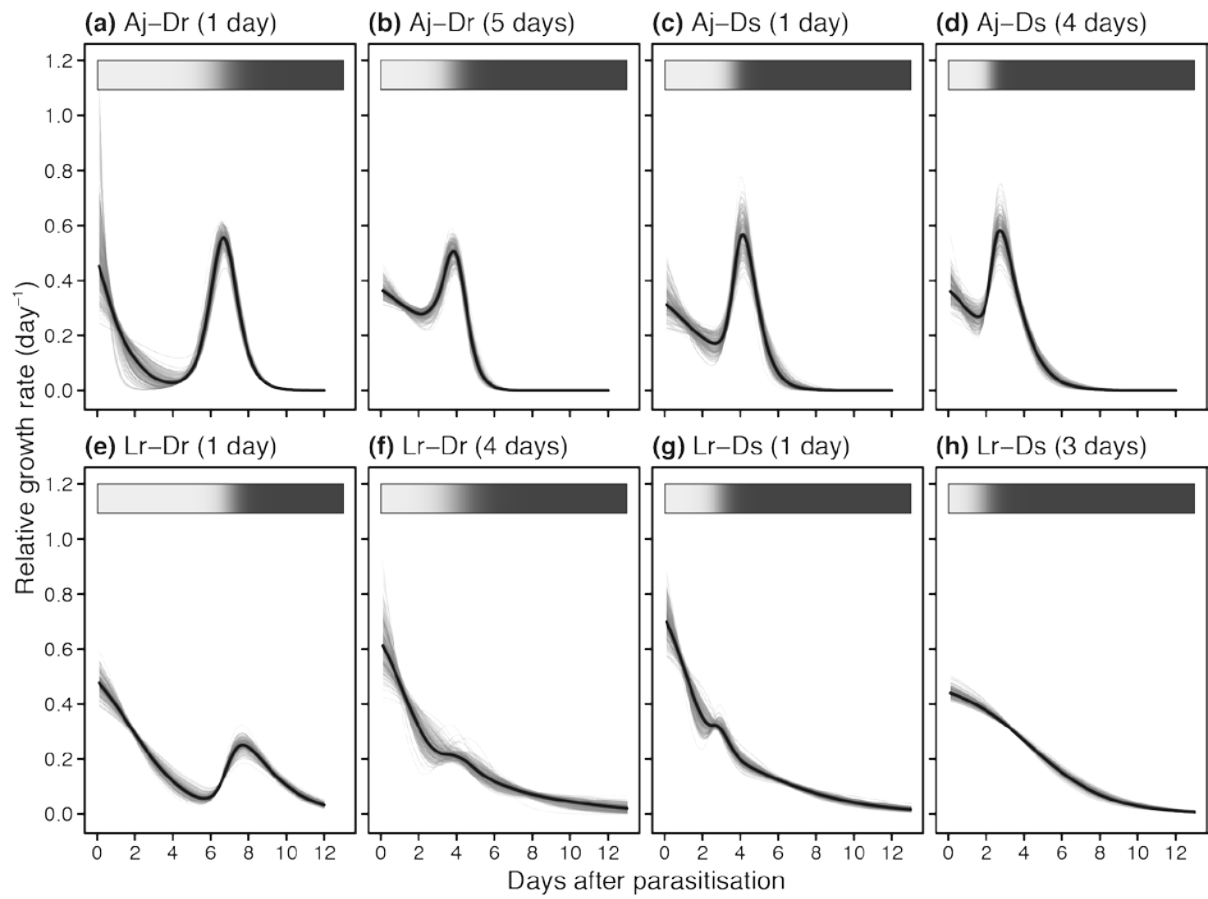


Figure 3