



Research

Cite this article: Khudr MS, Oldekop JA, Shuker DM, Preziosi RF. 2013 Parasitoid wasps influence where aphids die via an interspecific indirect genetic effect. *Biol Lett* 9: 20121151. <http://dx.doi.org/10.1098/rsbl.2012.1151>

Received: 12 December 2012

Accepted: 18 March 2013

Subject Areas:

ecology, evolution, behaviour

Keywords:

parasitoid, indirect genetic effects, interspecific indirect genetic effects, coevolution, pea aphid

Author for correspondence:

Mouhammad Shadi Khudr

e-mail: mouhammad.khudr@manchester.ac.uk

Evolutionary biology

Parasitoid wasps influence where aphids die via an interspecific indirect genetic effect

Mouhammad Shadi Khudr¹, Johan A. Oldekop^{1,2}, David M. Shuker³ and Richard F. Preziosi¹

¹Faculty of Life Sciences, University of Manchester, Michael Smith Building, Manchester, M13 9PT, UK

²Sheffield Institute for International Development, University of Sheffield, Sheffield, S1 4DP, UK

³School of Biology, University of St. Andrews, Harold Mitchell Building, St Andrews, Fife, KY16 9TH, UK

Host–parasite interactions are a key paradigm for understanding the process of coevolution. Central to coevolution is how genetic variation in interacting species allows parasites to evolve manipulative strategies. However, genetic variation in the parasite may also be associated with host phenotype changes, thereby changing the selection on both species. For instance, parasites often induce changes in the behaviour of their host to maximize their own fitness, yet the quantitative genetic basis for behavioural manipulation has not been fully demonstrated. Here, we show that the genotype of the parasitoid wasp *Aphidius ervi* has a significant effect on where its aphid host *Acyrtosiphon pisum* moves to die following parasitism, including the likelihood that the aphid abandons the plant. These results provide a clear example of an interspecific indirect genetic effect whereby the genetics of one species influences the expression of a specific behavioural trait in another.

1. Introduction

Parasite fitness often depends on host behaviour, and parasites often attempt to maximize fitness by host manipulation [1,2]. For example, parasites that require more than one host to complete their life cycle may cause changes in host behaviours that increase exposure to predators and heighten the chance of transmission to the final host [1–3]. Parasite-induced behaviours may also promote predator evasion responses of hosts [4] to avoid either premature transmission or death [1,5]. This sets up the potential for coevolution of parasite manipulation and host resistance traits [6–8].

Genetic variation is necessary for any evolutionary response to occur [6–9]. Parasite manipulation of hosts is a well-documented phenomenon. However, genetic variation of manipulation is rarely studied. Despite the development of indirect genetic effect (IGE) theory, which formalizes how the genotype of one individual can influence the phenotypic expression of a trait in another individual (from the same or different species) [10,11], there remain few empirical examples [12,13]. Host manipulation would represent an interspecific indirect genetic effect (IIGE) if different parasite genotypes differentially influenced host behaviour [11].

Parasitoid wasps attack a vast array of insects [14]. Behavioural modification of hosts by parasitoids is common [15–19], e.g. parasitoids can stimulate the aphids they attack to abandon their host plants [15–19], but see [20].

In *Aphidius ervi*, the life cycle begins with a female injecting one egg into the body cavity of its aphid host. The wasp larva consumes the host from the inside, leaving the exoskeleton to act as a puparium (mummy) for the larva. Aphid hosts display a range of defences [14,18,21,22] and infected aphids have been shown to differ in their mummification positions on their host plants [15,19].

Aphids bearing diapausing wasp larvae tend to abandon the plant and mummify in obscured sites [15,16], while individuals infested by non-diapausing larvae tend to leave the aphid colony to mummify on upper-leaf surfaces [15,16]. Here, we characterize variation in how a parasitoid manipulates its host, using IGE theory to gain insight into the potential evolutionary outcomes and to show how variation in host mummification position is determined by the genotype of the parasitoid.

2. Material and methods

Pea aphids undergo parthenogenesis during warm seasons in temperate regions and in controlled temperature conditions, making it is easy to maintain clonal groups in the laboratory. Individuals belonging to a single clone of the pea aphid *Acyrtosiphon pisum* Harris (Homoptera, Aphididae) were maintained on a continually replaced stock of broad beans (*Vicia faba*).

Aphidius ervi Haliday (Hymenoptera, Braconidae) is a parasitoid of various insects, including *A. pisum*. Two hundred and fifty wasps obtained from Koppert Systems (NL) were used to establish a colony that was maintained on pea aphids at 22 °C and a 16 L:8 D cycle. Emerging adult wasps were fed using cotton dental rolls soaked in 10 per cent sucrose solution. To control for maternal effects, the colony was maintained for one generation before the breeding experiments. To quantify the effect of exposure to parasitoid wasps on the distribution of aphids on or off the host plant, we first performed an experiment comparing the distribution of parasitized (13 caged plants) and non-parasitized aphids (10 caged plants). In a second experiment, we used a half-sib breeding design (for a haplodiploid system) to estimate the genetic variance components of the parasitoid's influence on: (i) where aphids formed mummies on the plant, (ii) if aphids remained on the plant, and (iii) the proportion of aphids that were parasitized. This design allows a separation of additive genetic variation from environmental and common environment (e.g. maternal) variation. We note that this experimental approach does not allow a distinction between the immediate effects of the parasitoid female on the aphids during parasitism and the effects of the developing larva after parasitism.

For the experiment, mummies containing wasp pupae were collected over several days, isolated individually in Petri dishes and kept in the dark at 4 °C in order to synchronize the eclosion of a large number of individuals. We randomly selected freshly eclosed male (sires) and female (dams) parents for breeding. Each mating pair was placed in a 00 pharmaceutical gelatine capsule. Thirteen sires were each mated to three dams immediately after eclosion, to generate a total of 13 half-sib and 39 full-sib families. Mated females were individually introduced into plastic cages with an aphid-proof mesh-top. Each cage contained a standardized broad bean plant (approx. five weeks old and 25 cm in height) infested with 30 juvenile aphids. Aphid mummies (containing wasp pupae) were collected after 9 days, isolated individually and maintained under the same growth conditions. At eclosion, four unmated daughters were selected from each family and placed individually into cages containing one *V. faba* plant (21 days old, 12 cm in height) infested with 30 juvenile aphids, yielding $N = 156$ (4×39) cages. Plants were divided by height into bottom (0–4 cm), middle (5–8 cm) and top (9–12 cm) thirds. At day 11, the positions of mummies were recorded on and off plant, and for each mummy on the plant, we recorded its position in the top, middle or bottom third of the plant.

Data were analysed in JMP 9 (SAS Inc). We compared the frequencies of mummies in each location using a nominal logistic regression model, with significance testing performed using maximum-likelihood and likelihood ratio tests (LRT). The

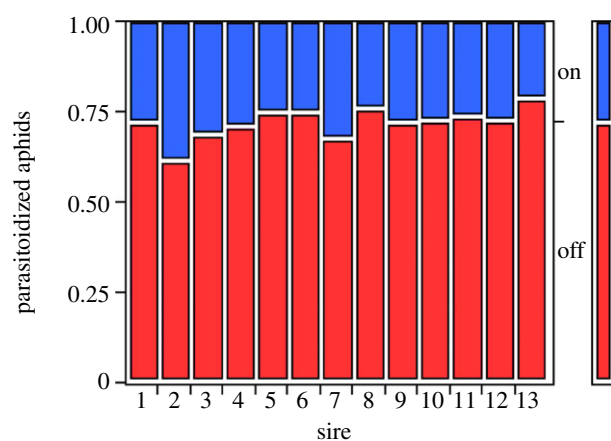


Figure 1. The proportion of mummified aphids on and off the plant for each wasp sire family.

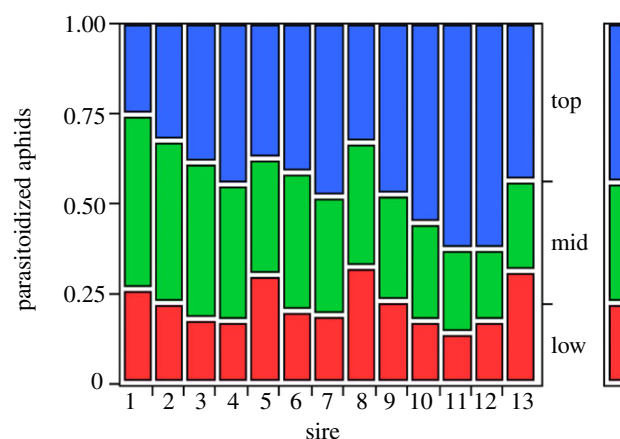


Figure 2. The proportion mummified aphids on each third of the plant for each wasp sire family.

quantitative genetic model used was a nested half-sib design, such that:

$$\text{mummified aphid position} = \text{sire} + \text{dam}(\text{sire}) + \text{daughter}[\text{dam}(\text{sire})] + \text{error}.$$

(Raw data have been deposited in Dryad, accession no.: <http://dx.doi.org/10.5061/dryad.kr62k>) [23].

3. Results

More aphids left their host plants when exposed to a parasitoid (73%) than when unexposed (9%: LRT, $\chi^2_1 = 561.5$, $p < 0.0001$). For aphids that remained on the plant, parasitism influenced the position on the plants where the mummies were found, with more parasitized individuals (mummies) found lower on the plants (LRT: $\chi^2_2 = 54.9$, $p < 0.0001$). We also found an IIGE; parasitoid wasp genotype was highly significantly associated with aphid behavioural modification. In the quantitative genetic experiment, there was a significant sire effect (additive genetic variation) on the likelihood that mummified aphids were found on or off the plant (figure 1), the position of mummified aphids on the plant (figure 2) and the level of parasitism (proportions of aphids parasitized; table 1). There were also significant dam effects for the wasps suggesting non-additive and/or maternal effects influencing parasitism rate and the behaviour of parasitized aphids (table 1).

Table 1. Effects of parasitoid wasp (sire, dam and daughter) on mummified aphid position and likelihood of parasitism. There were 13 sires, 33 dams and 125 daughters.

source	on versus off host plant		top, middle or bottom of host plant		parasitized versus non-parasitized	
	χ^2 (d.f.)	p-value	χ^2 (d.f.)	p-value	χ^2 (d.f.)	p-value
sire	28.85 (12)	0.0042	41.06 (24)	0.0164	58.21 (12)	<0.0001
dam	47.89 (13)	<0.0001	75.68 (40)	0.0006	42.51 (12)	<0.0001
daughter	183.89 (74)	<0.0001	328.58 (182)	0.00107	291.51 (41)	<0.0001

4. Discussion

We found that parasitoid attack can lead to behavioural modifications in an aphid host, with parasitoid genotype influencing this behavioural modification. This confirms that parasitoids can alter aphid behaviour, indicating a genetic basis for this trait in wasps [6,13–15,18], and a potential for wasp manipulation of their aphid host to evolve. Note that there was no genetic variation in aphids in our study and thus no information on the potential in the aphids to coevolve with the wasp.

Although genetic variation in the successful utilization of hosts (virulence) has been found before in parasitoids [6,7], this is the first time that variation in host modification has been associated with genetic variation in a parasitoid, providing an example of an IIGE [11].

Whether the overall change in location of the aphid mummies containing the wasp pupae following parasitism is to the benefit of the host or the parasite remains unclear, and in any case may well be context-dependent [9,15,21,22]. Nonetheless our data show that the extent of that change in behaviour is influenced by genetic variation in the wasps, part of which is non-additive in nature. The next step will be to uncover the molecular genetic basis of this manipulation, as shown by the recent discovery of a baculovirus gene that influences gypsy moth caterpillar climbing behaviour [24].

Although we have focused on the wasp, the behaviour of a parasitized aphid is perhaps best thought of as a shared phenotype of both the wasp and the aphid genomes [25], as envisaged by IGE theory [10,11]. Both selection and the genetic component of the phenotypes under selection become products of the interacting genotypes [9,10,22,25]. However, while the phenotype may be shared, there is an important asymmetry for understanding the evolutionary consequences of the parasitoid–host interaction [26]. For instance, parasitoids may have more choice of their aphid hosts than aphids have choice of their parasitoid attacker. This may bias how coevolution between host and parasitoid progresses [9,22,25,27].

Finally, given the economic value of aphid–parasitoid interactions in agro-ecosystems, our work suggests that genetic interactions across trophic levels may influence the success of bio-control attempts. We may well need to pay greater attention to what genotypes we release into such systems.

This study was funded by Damascus University in collaboration with The British Council (HECBP) to M.S.K. and through a small project grant from the British Ecological Society to J.A.O. D.M.S. is supported by an NERC Advanced Research Fellowship. We sincerely thank Dr Glen Powell at Imperial College (London) for providing the initial sample of *A. pisum* genotype (JF01/29).

References

- Moore J. 2002 *Parasites and the behavior of animals*. New York, NY: Oxford University Press.
- Poulin R. 2006 *Evolutionary ecology of parasites*. Princeton, NJ: Princeton University Press.
- Thomas F, Brodeur J, Maure F, Franceschi N, Blanchet S, Rigaud T. 2011 Intraspecific variability in host manipulation by parasites. *Infect. Genet. Evol.* **11**, 262–269. (doi:10.1016/j.meegid.2010.12.013)
- Hammerschmidt K, Koch K, Milinski M, Chub JC, Parker GA. 2009 When to go: optimization of host switching in parasites with complex life cycles. *Evolution* **63**, 1976–1986. (doi:10.1111/j.1558-5646.2009.00687.x)
- Levri EP. 1998 The influence of non-host predators on parasite-induced behavioral changes in a freshwater snail. *Oikos* **81**, 531–537. (doi:10.2307/3546773)
- Henter HJ. 1995 The potential for coevolution in a host–parasitoid system. II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution* **49**, 439–445. (doi:10.2307/2410268)
- Ferrari JC, Müller CB, Kraaijeveld AR, Godfray HJ. 2001 Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution* **55**, 1805–1814. (doi:10.1554/0014-3820(2001)055[1805:CVACIA]2.0.CO;2)
- Thompson JN. 1994 *The coevolutionary process*. Chicago, IL: University of Chicago Press.
- Carius HJ, Little TJ, Ebert D. 2001 Genetic variation in a host–parasite association: potential for coevolution and frequency-dependent selection. *Evolution* **55**, 1136–1145. (doi:10.1554/0014-3820(2001)055[1136:GVIAHP]2.0.CO;2)
- Moore AJ, Brodie ED, Wolf JB. 1997 Interacting phenotypes and the evolutionary process. I. Direct and indirect genetic effects of social interactions. *Evolution* **51**, 1352–1362. (doi:10.2307/2411187)
- Wolf JB. 2000 Indirect genetic effects and gene interactions. In *Epistasis and the evolutionary process* (eds JB Wolf, ED Brodie, MJ Wade), pp. 158–176. New York, NY: Oxford University Press.
- Bleakley BH, Wolf JB, Moore AJ. 2010 Quantitative genetics of social behavior. In *Social behaviour: genes, ecology and evolution* (eds T Szekely, J Komdeur, AJ Moore), pp. 29–54. Cambridge, UK: Cambridge University Press.
- Chenoweth SF, Rundle HD, Blows MW. 2010 Experimental evidence for the evolution of indirect genetic effects: changes in the interaction effect coefficient, ψ , due to sexual selection. *Evolution* **64**, 1849–1856. (doi:10.1111/j.1558-5646.2010.00952.x)
- Godfray HJ. 1994 *Parasitoids: behavioral and evolutionary ecology*. Princeton, NJ: Princeton University Press.
- Brodeur J, McNeil JN. 1989 Seasonal microhabitat selection by an endoparasitoid through adaptive

- modification of host behavior. *Science* **244**, 226–228. (doi:10.1126/science.244.4901.226)
16. Brodeur J, McNeil JN. 1992 Host behaviour modification by the endoparasitoid *Aphidius nigripes*: a strategy to reduce hyperparasitism. *Ecol. Entomol.* **17**, 97–104. (doi:10.1111/j.1365-2311.1992.tb01164.x)
 17. Brodeur J, Rosenheim JA. 2000 Intraguild interactions in aphid parasitoids. *Entomologia Experimentalis et Applicata* **97**, 93–108. (doi:10.1046/j.1570-7458.2000.00720.x)
 18. Wajnberg É, Bernstein C, Van Alphen J. 2008 *Behavioural ecology of insect parasitoids: from theoretical approaches to field applications*. Oxford, UK: Blackwell Publishing Ltd.
 19. Brodeur J, McNeil JN. 1990 Overwintering microhabitat selection by an endoparasitoid (Hymenoptera: Aphidiidae): induced phototactic and thigmokinetic responses in dying hosts. *J. Insect Behav.* **3**, 751–763. (doi:10.1007/BF01065963)
 20. Müller CB, Völkl V, Godfray HCJ. 1997 Are behavioural changes in parasitised aphids a protection against hyperparasitism? *Eur. J. Entomol.* **94**, 221–234.
 21. McAllister MK, Roitberg BD, Weldon KL. 1990 Adaptive suicide in pea aphids: decisions are cost sensitive. *Anim. Behav.* **40**, 167–175. (doi:10.1016/S0003-3472(05)80676-1)
 22. Smith Trail DR. 1980 Behavioral interactions between parasites and hosts: host suicide and the evolution of complex life cycles. *Am. Nat.* **116**, 77–91. (doi:10.1086/283612)
 23. Khudr MS, Oldekop JA, Shuker DM, Preziosi RF. 2013 Data from: parasitoid wasps influence where aphids die via an interspecific indirect genetic effect. *Dryad Digital Repository*. See <http://dx.doi.org/10.5061/dryad.kr62k>.
 24. Hoover K, Grove M, Gardner M, Hughes DP, McNeil J, Slavicek J. 2011 A gene for an extended phenotype. *Science* **333**, 1401. (doi:10.1126/science.1209199)
 25. Dawkins R. 1982 *The extended phenotype: the gene as the unit of selection*. Oxford, UK: W. H. Freeman & Company.
 26. Lapchin L, Guillemaud T. 2005 Asymmetry in host and parasitoid diffuse coevolution: when the red queen has to keep a finger in more than one pie. *Front. Zool.* **2**, 4. (doi:10.1186/1742-9994-2-4)
 27. Fritz RS. 1982 Selection for host modification by insect parasitoids. *Evolution* **36**, 283–288. (doi:10.2307/2408046)