



HAL
open science

Functional insights from the GC-poor genomes of two aphid parasitoids, *Aphidius ervi* and *Lysiphlebus fabarum*

Alice B. Dennis, Gabriel I. Ballesteros, Stéphanie Robin, Lukas Schrader, Jens Bast, Jan Berghöfer, Leo Beukeboom, Maya Belghazi, Anthony Bretaudeau, Jan Büllesbach, et al.

► To cite this version:

Alice B. Dennis, Gabriel I. Ballesteros, Stéphanie Robin, Lukas Schrader, Jens Bast, et al.. Functional insights from the GC-poor genomes of two aphid parasitoids, *Aphidius ervi* and *Lysiphlebus fabarum*. 2019. hal-02788571

HAL Id: hal-02788571

<https://hal.inrae.fr/hal-02788571>

Preprint submitted on 5 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 Functional insights from the GC-poor genomes of two aphid
2 parasitoids, *Aphidius ervi* and *Lysiphlebus fabarum*

3

4 Alice B. Dennis^{§1,2,3*}, Gabriel I. Ballesteros^{§4,5,6}, Stéphanie Robin^{7,8}, Lukas Schrader⁹, Jens
5 Bast^{10,11}, Jan Berghöfer⁹, Leo Beukeboom¹², Maya Belghazi¹³, Anthony Bretaudeau^{7,8}, Jan
6 Büllsbach⁹, Elizabeth Cash¹⁴, Dominique Colinet¹⁵, Zoé Dumas¹⁰, Patrizia Falabella¹⁶, Jean-
7 Luc Gatti¹⁵, Elzemie Geuverink¹², Joshua D. Gibson^{14,17}, Corinne Hertäg^{18,1}, Stefanie
8 Hartmann³, Emmanuelle Jacquin-Joly¹⁹, Mark Lammers⁹, Blas I. Lavandero⁶, Ina
9 Lindenbaum⁹, Lauriane Massardier-Galata¹⁵, Camille Meslin¹⁹, Nicolas Montagné¹⁹, Nina
10 Pak¹⁴, Marylène Poirié¹⁵, Rosanna Salvia¹⁶, Chris R. Smith²⁰, Denis Tagu⁷, Sophie Tares¹⁵,
11 Heiko Vogel²¹, Tanja Schwander¹⁰, Jean-Christophe Simon⁷, Christian C. Figueroa^{4,5},
12 Christoph Vorburger^{1,2}, Fabrice Legeai^{7,8}, and Jürgen Gadau⁹

13

14 § Joint first authors

15 *Author for correspondence: alicebdennis@gmail.com

16

17

18

19

20

21

22

¹ Department of Aquatic Ecology, Eawag, 8600 Dübendorf, Switzerland

² Institute of Integrative Biology, ETH Zürich, 8092 Zürich, Switzerland

³ Institute of Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany

⁴ Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile

⁵ Centre for Molecular and Functional Ecology in Agroecosystems, Universidad de Talca, Talca, Chile

⁶ Laboratorio de Control Biológico, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile

⁷ IGEPP, Agrocampus Ouest, INRA, Université de Rennes, 35650 Le Rheu, France

⁸ Université de Rennes 1, INRIA, CNRS, IRISA, 35000, Rennes, France

⁹ Institute for Evolution and Biodiversity, Universität Münster, Münster, Germany

¹⁰ Department of Ecology and Evolution, Université de Lausanne, 1015 Lausanne

¹¹ Institute of Zoology, Universität zu Köln, 50674 Köln

¹² Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands

¹³ Aix-Marseille Univ, CNRS, INP, Inst Neurophysiopathol, PINT, PFNT, Marseille, France

¹⁴ Department of Environmental Science, Policy, & Management, University of California, Berkeley, Berkeley, CA 94720, USA

¹⁵ Université Côte d'Azur, INRA, CNRS, ISA, Sophia Antipolis, France

¹⁶ University of Basilicata, Department of Sciences, 85100 Potenza, Italy

¹⁷ Department of Biology, Georgia Southern University, Statesboro, GA 30460, USA

¹⁸ D-USYS, Department of Environmental Systems Sciences, ETH Zürich, Switzerland

¹⁹ INRA, Sorbonne Université, CNRS, IRD, UPEC, Université Paris Diderot, Institute of Ecology and Environmental Sciences of Paris, iEES-Paris, F-78000 Versailles, France

²⁰ Department of Biology, Earlham College, Richmond, IN USA 47374

²¹ Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany

23 **Abstract**

24

25

26 **Background**

27 Parasitoid wasps have fascinating life cycles and play an important role in trophic
28 networks, yet little is known about their genome content and function. Parasitoids that
29 infect aphids are an important group with the potential for biocontrol, and infecting
30 aphids requires overcoming both aphid defenses and their defensive endosymbionts.

31

32 **Results**

33 We present the *de novo* genome assemblies, detailed annotation, and comparative
34 analysis of two closely related parasitoid wasps that target pest aphids: *Aphidius ervi*
35 and *Lysiphlebus fabarum* (Hymenoptera: Braconidae: Aphidiinae). The genomes are
36 small (139 and 141 Mbp), highly syntenic, and the most AT-rich reported thus far for
37 any arthropod (GC content: 25.8% and 23.8%). This nucleotide bias is accompanied by
38 skewed codon usage, and is stronger in genes with adult-biased expression. AT-richness
39 may be the consequence of reduced genome size, a near absence of DNA methylation,
40 and age-specific energy demands. We identify expansions of F-box/Leucine-rich-repeat
41 proteins, suggesting that diversification in this gene family may be associated with their
42 broad host range or with countering defenses from aphids' endosymbionts. The
43 absence of some immune genes (Toll and Imd pathways) resembles similar losses in
44 their aphid hosts, highlighting the potential impact of symbiosis on both aphids and
45 their parasitoids.

46

47 **Conclusions**

48 These findings are of fundamental interest for insect evolution and beyond. This will
49 provide a strong foundation for further functional studies including coevolution with
50 respect to their hosts, the basis of successful infection, and biocontrol. Both genomes
51 are available at <https://bipaa.genouest.org>.

52

53

54 **Keywords:** Parasitoid wasp, aphid host, *Aphidius ervi*, *Lysiphlebus fabarum*, GC content,
55 *de novo* genome assembly, DNA methylation loss, chemosensory genes, venom
56 proteins, Toll and Imd pathways

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71 Background

72 Parasites are ubiquitously present across all of life (Poulin 2007; Windsor 1998). Their
73 negative impact on host fitness can impose strong selection on hosts to resist, tolerate,
74 or escape potential parasites. Parasitoids are a special group of parasites whose
75 successful reproduction is fatal to the host (Godfray 1994; Quicke 2014). The
76 overwhelming majority of parasitoid insects are hymenopterans that parasitize other
77 terrestrial arthropods, and they are estimated to comprise up to 75% of the species-
78 rich insect order Hymenoptera (Forbes *et al.* 2018; Godfray 1994; Heraty 2009;
79 Pennacchio & Strand 2006). Parasitoid wasps target virtually all insects and
80 developmental stages (eggs, larvae, pupae, and adults), including other parasitoids
81 (Chen & van Achterberg 2018; Godfray 1994; Müller *et al.* 2004; Poelman *et al.* 2012).
82 Parasitoid radiations appear to have coincided with those of their hosts (Peters *et al.*
83 2017), and there is ample evidence that host-parasitoid relationships impose strong
84 reciprocal selection, promoting a dynamic process of antagonistic coevolution (Dupas
85 *et al.* 2003; Kraaijeveld *et al.* 1998; Vorburger & Perlman 2018).

86 Parasitoids of aphids play an economically important role in biological pest
87 control (Boivin *et al.* 2012; Heimpel & Mills 2017), and aphid-parasitoid interactions are
88 an excellent model to study antagonistic coevolution, specialization, and speciation
89 (Henter & Via 1995; Herzog *et al.* 2007). While parasitoids that target aphids have
90 evolved convergently several times, their largest radiation is found in the braconid
91 subfamily Aphidiinae, which contains at least 400 described species across 50 genera
92 (Chen & van Achterberg 2018; Shi & Chen 2005). As koinobiont parasitoids, their
93 development progresses initially in still living, feeding, and developing hosts, and ends
94 with the aphids' death and the emergence of adult parasitoids. Parasitoids increase

95 their success with a variety of strategies, including host choice (Chau & Mackauer 2000;
96 Łukasik *et al.* 2013), altering larval development timing (Martinez *et al.* 2016), injecting
97 venom during stinging and oviposition, and developing special cells called teratocytes
98 (Burke & Strand 2014; Colinet *et al.* 2014; Falabella *et al.* 2003; Poirié *et al.* 2014; Strand
99 2014). In response to strong selection imposed by parasitoids, aphids have evolved
100 numerous defenses, including behavioral strategies (Gross 1993), immune defenses
101 (Schmitz *et al.* 2012), and symbioses with heritable endosymbiotic bacteria whose
102 integrated phages can produce toxins to hinder parasitoid success (Oliver *et al.* 2010;
103 Oliver & Higashi 2018; Vorburger & Perlman 2018).

104 The parasitoid wasps *Lysiphlebus fabarum* and *Aphidius ervi* (*Braconidae*:
105 Aphidiinae) are closely related endoparasitoids (Figure 1). In the wild both species are
106 found infecting a wide range of aphid species although their host ranges differ, with *A.*
107 *ervi* more specialized on aphids in the Macrosiphini tribe and *L. fabarum* on the Aphidini
108 tribe (Kavallieratos *et al.* 2004; Monticelli *et al.* 2019). In both taxa, there is evidence
109 that parasitoid success is hindered by the presence of defensive symbionts in the aphid
110 haemocoel, including the bacteria *Hamiltonella*, *Regiella*, and *Serratia* (Oliver *et al.*
111 2003; Vorburger *et al.* 2010). Studies employing experimental evolution in both species
112 have shown that wild-caught populations can counter-adapt to cope with aphids and
113 the defenses of their endosymbionts, and that the coevolutionary relationships
114 between parasitoids and the aphids' symbionts likely fuel diversification of both
115 parasitoids and their hosts (Dennis *et al.* 2017; Dion *et al.* 2011; Rouchet & Vorburger
116 2014). While a number of parasitoid taxa are known to inject viruses and virus-like
117 particles into their hosts, there is thus far no evidence that this occurs in parasitoids
118 that target aphids; emerging studies have identified abundant RNA viruses in *L.*

119 *fabarum* (Lüthi *et al.* submitted; Obbard *et al.* in revision), but whether this impacts
120 their ability to parasitize is not yet fully understood.

121 These two closely related parasitoids differ in several important life history
122 traits, and are expected to have experienced different selective regimes as a result.
123 *Aphidius ervi* is has successfully been introduced widely (Nearctic, Neotropics) as a
124 biological control agent (far more than *L. fabarum*). Studies on both native and
125 introduced populations of *A. ervi* have shown ongoing evolutionary processes with
126 regard to host preferences, gene flow, and other life history components (Henry *et al.*
127 2008; Hufbauer *et al.* 2004; Zepeda-Paulo *et al.* 2015; Zepeda-Paulo *et al.* 2013). *A. ervi*
128 is known to reproduce only sexually, whereas *L. fabarum* is capable of both sexual and
129 asexual reproduction. In fact, wild *L. fabarum* populations are more commonly
130 composed of asexually reproducing (thelytokous) individuals (Sandrock *et al.* 2011). In
131 asexual populations, diploid *L. fabarum* females produce diploid female offspring via
132 central fusion automixis (Belshaw & Quicke 2003). While they are genetically
133 differentiated, sexual and asexual populations appear to maintain gene flow and thus
134 both reproductive modes and genome-wide heterozygosity are maintained in the
135 species as a whole (Mateo Leach *et al.* 2009; Sandrock *et al.* 2011; Sandrock &
136 Vorburger 2011). *Aphidius. ervi* and *L. fabarum* are also expected to have experienced
137 different selective regimes with regard to their cuticular hydrocarbon profiles and
138 chemosensory perception. *Lysiphlebus* target aphid species that are ant-tended, and
139 ants are known to prevent parasitoid attacks on “their” aphids (Rasekh *et al.* 2010). To
140 counter ant defenses, *L. fabarum* has evolved the ability to mimic the cuticular
141 hydrocarbon profile of the aphid hosts (Liepert & Dettner 1993, 1996). With this, they
142 are able to circumvent ant defenses and access this challenging ecological niche, from

143 which they also benefit nutritionally; they are the only parasitoid species thus far
144 documented to behaviorally encourage aphid honeydew production and consume this
145 high-sugar reward (Rasekh *et al.* 2010; Völkl 1992; Völkl 1997).

146 We present here the genomes of *A. ervi* and *L. fabarum*, assembled *de novo*
147 using a hybrid sequencing approach. The two genomes are highly syntenic and strongly
148 biased towards AT nucleotides. We have examined GC content in the context of host
149 environment, nutrient limitation, and gene expression. By comparing these two
150 genomes we identify key functional specificities in genes underlying venom
151 composition, oxidative phosphorylation, cuticular hydrocarbon composition, and
152 chemosensory perception. In both species, we identify losses in key immune genes and
153 an apparent lack of key DNA methylation machinery. These are functionally important
154 traits associated with success infecting aphids and the evolution of related traits across
155 all of Hymenoptera.

156

157 **Results and Discussion**

158 ***Two de novo genome assemblies***

159 The genome assemblies for *A. ervi* and *L. fabarum* were constructed using hybrid
160 approaches that incorporated high-coverage short read (Illumina) and long-read (Pac
161 Bio) sequencing, but were assembled with different parameters (Supplementary Tables
162 1, 2). This produced two high quality genome assemblies (*A. ervi* N50 = 581kb, *L.*
163 *fabarum* N50 = 216kb) with similar total lengths (*A. ervi*: 139MB, *L. fabarum*: 141MB)
164 but different ranges of scaffold-sizes (Table 1, Supplementary Table 3). These assembly
165 lengths are within previous estimates of 110-180Mbp for braconids, including *A. ervi*
166 (Ardila-Garcia *et al.* 2010; Hanrahan & Johnston 2011). Both assemblies are available

167 in NCBI (SAMN13190903-4) and can be accessed via the Bioinformatics Platform for
 168 Agroecosystem Arthropods (BIPAA, <https://bipaa.genouest.org>), which contains the
 169 full annotation reports, predicted genes, and can be searched via both keywords and
 170 blast.

171 We constructed linkage groups for the *L. fabarum* scaffolds using phased SNPs
 172 from the haploid (male) sons of a single female wasp from a sexually reproducing
 173 population. This placed the 297 largest scaffolds (>50% of the nucleotides,
 174 Supplementary Table 5, Supplementary Figure 1, Additional File 1) into the expected
 175 six chromosomes (Belshaw & Quicke 2003). With this largely contiguous assembly, we
 176 show that the two genomes are highly syntenic, with >60k links in alignments made by
 177 NUCmer (Kurtz *et al.* 2004) and >350 large syntenic blocks that match the six *L. fabarum*
 178 chromosomes to 28 *A. ervi* scaffolds (Supplementary Figures 2 and 3).

179

180 *Table 1: Assembly and draft annotation statistics*

	<i>A. ervi</i>	<i>L. fabarum</i>
Assembly statistics		
Total length (bp)	138,951,524	140,705,580
Longest scaffold (bp)	3,671,467	2,183,677
scaffolds	5,778	1,698
scaffolds \geq 3,000 bp	1,503	1,698
N50 (bp)	581,355	216,143
GC %	25.8%	23.8%
Annotation statistics		
Exons	95,322	74,701
Introns	74,978	59,498
CDS	20,344	15,203
% genome covered by CDS	17.8%	14.9%
GC % in CDS	31.9%	29.8%
GC % of 3 rd position in CDS	15.5%	10.7%
CDS with transcriptomic support	77.8%	88.3%

181

182

183 Within the two assemblies, we used the Maker2 annotation pipeline to predict
184 coding genes (CDS) for the two genomes, and these were functionally annotated
185 against the NCBI *nr* database (NCBI), matches to gene ontology (GO) terms, and
186 predictions for known protein motifs, signal peptides, and transmembrane domains
187 (Supplemental Table 6). In *A. ervi* there were 20,344 predicted genes comprising
188 27.8Mbp, while in *L. fabarum* there were 15,203 genes across 21.9 Mbp (Table 1).
189 These numbers are on par with those predicted in other hymenopteran genomes
190 (Table 2), and comparisons among taxa suggest that the lower number of predicted
191 genes in *L. fabarum* are more likely due to their loss than to a gene gain in *A. ervi*.
192 However, it is important to recognize that predictive annotation is imperfect and any
193 missing genes should be specifically screened with more rigorous methods. In both
194 species, there was high transcriptomic support for the predicted genes (77.8% in *A. ervi*
195 and 88.3% in *L. fabarum*). The two genome annotations appear to be largely complete;
196 at the nucleotide level, we could match 94.8% (*A. ervi*) and 76.3% (*L. fabarum*) of the
197 1,658 core orthologous BUSCO genes for Insecta in both species (Supplementary Table
198 4). Within the predicted genes, protein-level matches to the BUSCO genes were
199 improved in *L. fabarum* (95.9%) and slightly lower for *A. ervi* (93.7%). These numbers
200 suggest that low GC content did not negatively impact gene prediction (Supplementary
201 Table 4).

202 A survey of transposable Elements (TEs) identified a similar overall number of
203 putative TE elements in the two assemblies (*A. ervi*: 67,695 and *L. fabarum*: 60,306,
204 Supplementary Table 7). Despite this similarity, the overall genomic coverage by TEs is
205 larger in *L. fabarum* (41%, 58 Mbp) than in *A. ervi* (22%, 31 Mbp) and they differ in the
206 TE classes that they contain (Supplementary Table 7, Supplementary Figures 4, 5). The

207 spread of reported TE coverage in arthropods is quite large, even among *Drosophila*
 208 species (ca. 2.7% - 25%, *Drosophila* 12 Genomes *et al.* 2007). Within parasitoids,
 209 reported TE content also varies, and relatively low coverage in the parasitoid
 210 *Macrocentrus cingulum* in comparison to *Nasonia vitripennis* (24.9% vs 40.6% Yin *et al.*
 211 2018) was attributed the smaller genome size of *M. cingulum* (127.9Mbp and
 212 295.7Mbp, respectively, Table 3). However, the variation we observe here suggests
 213 that differences in predicted TE content may be evolutionary quite labile, even within
 214 closely related species with the same genome size.

215

216 *Table 2: Assembly summary statistics compared to other parasitoid genomes. All species are from the family*
 217 *Braconidae, except for N. vitripennis (Pteromalidae). Protein counts from the NCBI genome deposition.*

Parasitoid species	Assembly	Total Length (Mbp)	Scaffold Count	Scaffold N50 (bp)	Predicted genes (CDS)	GC (%)	NCBI BioProject
<i>Aphidius ervi</i>	A. ervi_v3	139.0	5,778	581,355	20,344	25.8	<i>This paper</i>
<i>Lysiphlebus fabarum</i>	L. fabarum_v1	140.7	1,698	216,143	15,203	23.8	<i>This paper</i>
<i>Fopius arisanus</i>	ASM80636v1	153.6	1,042	51,867	18,906	39.4	PRJNA258104 (Geib <i>et al.</i> 2017)
<i>Diachasma alloeum</i>	Dall1.0	388.8	3,968	44,932	19,692	39.1	PRJNA284396 (Tvedte <i>et al.</i> 2019)
<i>Microplitis demolitor</i>	Mdem 2	241.2	1,794	27,508	18,586	33.1	PRJNA251518 (Burke <i>et al.</i> 2018)
<i>Cotesia vestalis</i>	ASM95615v1	186.1	9,156	46,055	-	30.4	PRJNA271135
<i>Macrocentrus cingulum</i>	MCINOGS1.0	127.9	12,056	65,089	11,993	35.6	PRJNA361069 (Yin <i>et al.</i> 2018)
<i>Nasonia vitripennis</i>	Nvit_2.1	295.7	6,169	18,840	24,891	40.6	PRJNA13660 (Werren <i>et al.</i> 2010)

218

219 *GC content*

220 The *L. fabarum* and *A. ervi* genomes are the most GC-poor of insect genomes
 221 sequenced to date (GC content: 25.8% and 23.8% for *A. ervi* and *L. fabarum*,
 222 respectively, Table 3, Supplementary Figure 6). This nucleotide bias is accompanied by
 223 strong codon bias in the predicted genes, meaning that within the possible codons for

224 each amino acid, the two genomes are almost universally skewed towards the codon(s)
225 with the lowest GC content (measured as Relative Synonymous Codon Usage, RSCU,
226 Figure 2). These patterns are much more extreme than RSCU found in other
227 hymenopterans, which are known to prefer codons that end in –A or –U (Behura &
228 Severson 2013). This codon bias has functional consequences; work in other taxa has
229 shown that codon usage is tied to both expression efficiency and mRNA stability
230 (Barahimipour *et al.* 2015).

231 Low GC content could be a consequence of the relatively small size of these
232 genomes. Genome size and GC content are positively correlated in a diverse set of taxa
233 including bacteria (Almpanis *et al.* 2018; McCutcheon *et al.* 2009), plants (Šmarda *et al.*
234 2014; Veleba *et al.* 2016), and vertebrates (Vinogradov 1998). This widespread pattern
235 may be driven by GC-rich repetitive elements that are more abundant in larger
236 genomes, stronger selection on thermal stability in larger genomes, or thermal stability
237 associated with the environment (Šmarda *et al.* 2014; Vinogradov 1998). The apparent
238 lack of DNA methylation in this system may also contribute to low GC content (see
239 below and Bewick *et al.* 2017). Methylation is a stabilizing factor with regard to GC
240 content (Mugal *et al.* 2015), so its absence could relax selection on GC content and
241 allow it to decline. However, neither the absence of methylation nor codon bias are
242 unique to these taxa, suggesting that some additional selective factors or genetic drift
243 may have further shaped the composition of these two genomes.

244 We used two approaches to investigate whether environmental constraints
245 could drive extremely low GC content, but found no evidence for such constraints.
246 There is reason to expect that environment could contribute to the low GC content of
247 these genomes; in taxa including bacteria (Foerstner *et al.* 2005) and plants (Šmarda *et*

248 *al.* 2014) the environment has been shown to influence GC content via limitation in
249 elements including nitrogen. These two wasps parasitize aphids exclusively, and aphids
250 themselves have relatively low genome-wide GC content. This includes the pea aphid
251 (*Acyrtosiphon pisum*), which is a frequent host of *A. ervi* and also has notably low GC
252 content (29.8%, Li *et al.* 2019). This is not limited to *A. pisum*, with other aphid
253 genomes' GC content ranging between 26.8% - 30% (Additional File 2), perhaps related
254 to their high-sugar, low-nitrogen, sap diet. One way to explore the restrictions imposed
255 by nutrient limitation is to look at the expressed genes, since selective pressure should
256 be higher for genes that are more highly expressed (Ran & Higgs 2010; Seward & Kelly
257 2016). For our first test, we explored potential constraints in the most highly expressed
258 genes in both genomes. In both species, the most highly expressed 5% of genes had
259 higher GC content and higher nitrogen content, although the higher number of
260 nitrogen molecules in G's and C's means that these two measures cannot be entirely
261 disentangled (Additional File 3, Supplementary Figure 7). This is in line with
262 observations across many taxa, and with the idea that GC-rich mRNA has increased
263 expression via its stability and secondary structure (Kudla *et al.* 2009; Plotkin & Kudla
264 2011). For a second approach to examining constraints, we compared codon usage
265 between our genomes and taxa associated with this parasitoid-host-endosymbiont
266 system (Supplementary Table 8). We found no evidence of similarity in codon usage
267 (scaled as RSCU) nor in nitrogen content (scaled per amino acid) between parasitoids
268 and host aphids, the primary endosymbionts *Buchnera* nor, with the secondary
269 endosymbiont *Hamiltonella* (Supplementary Figures 8-10). Together, these tests do not
270 support environmental constraints as the driver of low GC content in these two
271 genomes.

272 In contrast, we did find evidence for reduced GC content in genes expressed at
273 different parasitoid life-history stages. We found higher GC content in larvae-biased
274 genes in *L. fabarum* (Figure 3). This was true when we compared the 10% most highly
275 expressed genes in adults (32.6% GC) and larvae (33.2%, $p=1.2e-116$, Figure 3,
276 Additional File 3), and this pattern holds even more strongly for genes that are
277 differentially expressed between adults (upregulated in adults: 28.7% GC) and larvae
278 (upregulated in larvae: 30.7% GC, $p=2.2e-80$. Note that the most highly expressed
279 genes overlap partially with those that are differentially expressed, Additional File 3).
280 At the same time, we found no evidence that nitrogen content differs in either of these
281 comparisons (Figure 3). While the magnitude of these differences is not very large,
282 subtle differences in gene content are hypothesized to be the result of selection in
283 other systems (Acquisti *et al.* 2009). It seems plausible that GC content differences
284 among genes expressed at different life history stages could be selected in a process
285 analogous to the small changes in gene expression that are linked to large phenotypic
286 differences within and between species (Romero *et al.* 2012). One explanation for
287 lower GC content in adult-biased genes could be differences in energy demands and
288 availability of resource across life stages. Given the extreme codon bias in these
289 genomes (Figure 2), using codons that match this bias is expected to be more efficient
290 and accurate, resulting in lower energy consumption and faster turnover (Chaney &
291 Clark 2015; Galtier *et al.* 2018; Kudla *et al.* 2006; Rao *et al.* 2013). Expressing AT-rich
292 genes is slightly more energy-efficient in itself, and this could favor otherwise neutral
293 mutations from GC to AT (Rocha & Danchin 2002). There is good motivation for adults
294 to have a greater demand for energy efficiency. Adult parasitoids usually feed on
295 carbohydrate rich but protein and lipid poor resources like nectar, while performing

296 costly tasks including flying, mating, and laying eggs. Meanwhile, parasitoid larvae are
297 feeding on their aphid host's tissue, and likely benefit further from nutrients coming
298 from the aphids' endosymbionts, while their only task is to grow as fast as possible
299 (Cheng *et al.* 2011; Miao *et al.* 2004; Pennacchio *et al.* 1999).

300 This supports the idea that selection at the level of gene expression is shaping
301 the GC content of these genomes. Nonetheless, further work should more explicitly
302 test both nutrient limitation and how selective pressures differ across life-history
303 stages. While we do not have the power to test for GC-biased gene conversion with
304 two taxa, the even lower third position GC content (15.5% and 10.7%, Table 1) suggests
305 that this should be tested in relation to other parasitoids (Galtier *et al.* 2018). Further
306 explanations to be considered include effective population size, translational efficiency,
307 and mutational bias (Behura & Severson 2013; Bentele *et al.* 2013; Galtier *et al.* 2018).
308 Altogether, these patterns raise important questions about how codon biases impact
309 genome content, and whether synonymous mutations are always functionally neutral
310 (Plotkin & Kudla 2011; Powell & Moriyama 1997).

311

312 *Orphan genes in the assembly*

313 To examine genes that may underlie novel functional adaptation, we identified
314 sequences that are unique within the predicted genes in the *A. ervi* and *L. fabarum*
315 genomes. We defined orphan genes as predicted genes with transcriptomic support
316 and with no identifiable homology based on searches against the NCBI *nr*, *nt*, and
317 Swissprot databases. With this, we identified 2,568 (*A. ervi*, Additional File 4) and 968
318 (*L. fabarum*, Additional File 5) putative orphans (Supplementary Table 9). The
319 evolutionary origin of these orphan genes is not known (Gold *et al.* 2018; Van Oss &

320 Carvunis 2019), but their retention or evolution could be important to understanding
321 specific functions or traits in these taxa. The higher number of orphan genes in *A. ervi*
322 partially explains the absolute difference in the number of annotated genes between
323 both taxa.

324

325 ***Gene family expansions***

326 To examine gene families that may have undergone expansions in association with
327 functional divergence and specialization, we identified groups of orthologous genes
328 that have increased and decreased in size in the two genomes, relative to one another.
329 We identified these species-specific gene-family expansions using the OMA standalone
330 package (Altenhoff *et al.* 2018). OMA predicted 8,817 OMA groups (strict 1:1 orthologs)
331 and 8,578 HOGs (Hierarchical Ortholog Groups, Additional File 6). Putative gene-family
332 expansions would be found in the predicted HOGs, because they are calculated to allow
333 for >1 member per species. Among these, there were more groups in which *A. ervi*
334 possessed more genes than *L. fabarum* (865 groups with more genes in *A. ervi*, 223
335 with more in *L. fabarum*, Supplementary Figure 11, Additional File 6). To examine only
336 the largest gene-family expansions, we looked further at the HOGs containing >20
337 genes (10 HOG groups, Supplementary Figure 12). Strikingly, the four largest
338 expansions were more abundant in *A. ervi* and were all identified as F-box proteins/
339 Leucine-rich-repeat proteins (*LRR*, total: 232 genes in *A. ervi* and 68 in *L. fabarum*,
340 Supplementary Figure 12, Additional File 6). This signature of expansion does not
341 appear to be due to fragmentation in the *A. ervi* assembly: the size of scaffolds
342 containing *LRRs* is on average larger in *A. ervi* than in *L. fabarum* (Welch two-sampled
343 t-test, $p=0.001$, Supplementary Figure 13).

344 The *LRRs* are a broad class of proteins associated with protein-protein
345 interactions, including putative venom components in these parasitoids (Colinet *et al.*
346 2014). *LRRs* belong to a larger category of leucine rich repeat pattern recognition
347 receptor proteins, which are an important component of innate immunity and cell-
348 surface recognition of bacterial intruders and include toll-like receptors in insects
349 (Soanes & Talbot 2010; Takeda & Akira 2005). While the functions of these proteins are
350 diverse, expansion in F-box/*LRR* proteins has been shown to have specific function in
351 immunity in parasitic insects. In the Hessian fly (*Mayetiola destructor*), fly-encoded F-
352 box/*LRR* proteins bind with plant-encoded proteins to form a complex that blocks the
353 plant's immune defenses against the parasitic fly (Zhao *et al.* 2015). Thus, we
354 hypothesize that this class of proteins has expanded in these parasitoids in relation to
355 recognizing the diverse bacterial defenses of their aphid hosts. Under this hypothesis,
356 we argue that expansion of F-box/*LRR* proteins contributes to the broad host
357 recognition in both species, and that their greater abundance in *A. ervi* may be
358 associated with a recent arms race with respect to the immune defenses and protective
359 endosymbionts of their host aphids.

360 The six largest gene families that were expanded in *L. fabarum*, relative to *A.*
361 *ervi*, were less consistently annotated. Interestingly, they contained two different
362 histone proteins: Histone H2B and H2A (Supplementary Figure 12). All eukaryotic
363 genomes examined to date contain multiple histone genes for the same histone
364 variants found in humans (e.g. 22 genes for H2B or 16 genes for H2A in humans, Singh
365 *et al.* 2018), and it has recently been suggested that these histone variants are not
366 functionally equivalent but rather play a role in chromatin regulation (Singh *et al.* 2018).
367 Hence, these variants could also play a role in several *L. fabarum* specific traits,

368 including the switch from sexual to asexual reproduction (thelytoky); in mammals, sex
369 determination has been linked to regulation via histone modification (Kuroki *et al.*
370 2013).

371

372 **Venom proteins**

373 Venom injected at oviposition is crucial for successful reproduction in most parasitoid
374 wasp species (Moreau & Asgari 2015; Poirié *et al.* 2014). The venom of *A. ervi* was
375 previously analyzed using a combined transcriptomic and proteomic approach (Colinet
376 *et al.* 2014), and we applied similar methods here to compare the venom composition
377 in *L. fabarum*. The venom gland in *L. fabarum* is morphologically different from *A. ervi*
378 (Supplementary Figure 14). A total of 35 *L. fabarum* proteins were identified as putative
379 venom proteins using 1D gel electrophoresis and mass spectrometry, combined with
380 transcriptomic and the genome data (Supplementary Figure 15, Additional File 7,
381 Dennis *et al.* 2017). These putative venom proteins were identified based on predicted
382 secretion (for complete sequences) and the absence of a match to typical cellular
383 proteins (e.g. actin, myosin). To match the analysis between the two taxa, the previous
384 *A. ervi* venom data (Colinet *et al.* 2014) was analyzed using the same criteria as *L.*
385 *fabarum*. This identified 32 putative venom proteins in *A. ervi* (Additional File 7).

386 Although these two species differ in their host range (Kavallieratos *et al.* 2004),
387 comparison of venom proteins between species revealed that more than 50% of the
388 proteins are shared between species (Figure 4A and Additional File 7), corresponding
389 to more than 70% of the putative function categories that were predicted (Figure 4B
390 and Additional File 7). Among venom proteins shared between both parasitoids, a
391 gamma glutamyl transpeptidase (GGT1) is the most abundant protein in the venom of

392 both *A. ervi* (Colinet *et al.* 2014) and *L. fabarum* (Additional File 7). This protein has
393 been suggested to be involved in the castration of the aphid host after parasitism
394 (Falabella *et al.* 2007). As previously reported for *A. ervi* (Colinet *et al.* 2014), a second
395 GGT venom protein (GGT2) containing mutations in the active site was also found in
396 the venom of *L. fabarum* (Supplementary Figure 16, 17). Phylogenetic analysis (Figure
397 5) revealed that the *A. ervi* and *L. fabarum* GGT venom proteins occur in a single clade
398 in which GGT1 venom proteins group separately from GGT2 venom proteins, thus
399 suggesting that they originated from a duplication that occurred prior to the split from
400 their most recent common ancestor. As previously shown for *A. ervi*, the GGT venom
401 proteins of *A. ervi* and *L. fabarum* are found in one of the three clades described for
402 the non-venomous hymenopteran GGT proteins (clade "A", Figure 5 and Colinet *et al.*
403 2014). Within this clade, venomous and non-venomous GGT proteins had a similar exon
404 structure, except for exon 1 that corresponds to the signal peptide only present in
405 venomous GGT proteins (Supplementary Figure 17). *Aphidius ervi* and *L. fabarum*
406 venomous GGT proteins thus probably result from a single imperfect duplication of the
407 non-venomous GGT gene belonging to clade A in their common ancestor, followed by
408 recruitment of the signal peptide coding sequence. This first imperfect duplication
409 event would then have been followed by a second duplication of the newly recruited
410 venomous GGT gene before the separation of both species.

411 The presence of truncated *LRR* proteins was previously reported in venom of *A.*
412 *ervi* (Colinet *et al.* 2014) and other Braconidae (Mathé-Hubert *et al.* 2016) that likely
413 interfere with the host immune response. Several *LRR* proteins were found in the
414 venom of *L. fabarum* as well, however these results should be interpreted with caution
415 since the sequences were incomplete and the presence of a signal peptide could not

416 be confirmed (Additional File 7). Moreover, these putative venom proteins were only
417 identified from transcriptomic data of the venom apparatus and we could not find any
418 corresponding annotated gene in the genome. This supports the idea that gene-family
419 expansions in putative F-box/*LRR* proteins (discussed above) are not related to venom
420 production.

421 Approximately 50% of the identified venom proteins were unique to either *A.*
422 *ervi* or *L. fabarum*, and these could be related to their differing host ranges (Additional
423 File 7). However, most of these proteins had no predicted function, making it difficult
424 to hypothesize their possible role in parasitism success. Among the venom proteins
425 with a predicted function, an apolipophorin was found in the venom of *L. fabarum* but
426 not in *A. ervi*. Apolipophorin is an insect-specific apolipoprotein involved in lipid
427 transport and innate immunity that is not commonly found in venoms. Among
428 parasitoid wasps, apolipophorin has been described in the venom of the ichneumonid
429 *Hyposoter didymator* (Dorémus *et al.* 2013) and the encyrtid *Diversinervus elegans* (Liu
430 *et al.* 2017), but its function is yet to be deciphered. Apolipophorin is also present in
431 low abundance in honeybee venom where it could have antibacterial activity (Kim & Jin
432 2015; Van Vaerenbergh *et al.* 2014). Lastly, we could not find *L. fabarum* homologs for
433 any of the three secreted cystein-rich toxin-like peptides that are highly expressed in
434 the *A. ervi* venom apparatus (Additional File 7). However, this may not be definitive
435 since the search for similarities in the genome is complicated by the small size of these
436 toxin-like sequences.

437

438

439

440 *Table 3: Summary of manual curations of select gene families in the two parasitoid genomes*

Category	<i>A. ervi</i>	<i>L. fabarum</i>
Venom proteins	32	35
Desaturases*	16	15
Immune genes†	216	216
Osiris genes	21	25
Mitochondrial Oxidative Phosphorylation System (OXPHOS)**	75	74
Chemosensory group		
Chemosensory: Odorant receptors (ORs)	228	156
Chemosensory: Ionotropic chemosensory receptors (IRs)	42	40
Chemosensory: Odorant-binding proteins (OBPs)	14	14
Chemosensory: Chemosensory proteins (CSPs)	11	13
Sex determination group		
Sex determination: Core (transformer, doublesex)	4	3
Sex determination: Related genes	6	5
DNA methylation genes	2	2
TOTALS	667	598

441 *Note 1: Includes genes that are partial, ambiguous, or potential pseudogenes †Note2: although the same number,
442 the set of immune genes is not identical in the two genomes.

443

444

445 **Key gene families**

446 We manually annotated more than 1,000 genes (667 for *A. ervi* and 598 for *L. fabarum*;

447 Table 3) using Apollo, hosted on the BIPAA website (Dunn *et al.* 2019;

448 <https://bipaa.genouest.org> ; Lee *et al.* 2013) to confirm and improve the results of the

449 machine annotation. This is especially important for large gene families, which are

450 usually poorly annotated by automatic prediction (Robertson *et al.* 2018); since such

451 gene families potentially underlie key adaptive differences between the two

452 parasitoids, accurate annotation is needed.

453

454 **Desaturases**

455 Desaturases are an important gene family that introduce carbon-carbon double bonds

456 in fatty acyl chains in insects (Los & Murata 1998; Sperling *et al.* 2003). While these

457 function broadly across taxa, a subset of these genes (specifically acyl-CoA desaturases)
458 have been implicated in insect chemical recognition for roles including alkene
459 production and modification of fatty acids (Helmkampf *et al.* 2015). This gene family is
460 particularly interesting because it has been shown that *Lysiphlebus cardui*, a close
461 relative of *L. fabarum*, have no unsaturated cuticular hydrocarbons, just as is seen in its
462 aphid host. This allows the parasitoid to go undetected in aphid colonies that are ant-
463 tended and therefore better parasitize them (Liepert & Dettner 1996). We confirmed
464 that the same is true for *L. fabarum*; its CHC profile is dominated by saturated
465 hydrocarbons (alkanes), contains only trace alkenes, and is completely lacking dienes
466 (Supplementary Figure 18, 20). In contrast, *A. ervi* females produce a large amount of
467 unsaturated hydrocarbons, with a significant amount of alkenes and alkadienes in their
468 CHC profiles (app. 70% of the CHC profile are alkenes/alkadienes, Supplementary
469 Figure 19, 20).

470 The loss of one annotated desaturase gene in *L. fabarum* compared to *A. ervi*
471 (Table 3) might explain these differences in the composition of their CHC profiles,
472 especially their apparent inability to synthesize dienes. We also note there is little
473 evidence that members of this gene family are clustered in the genome (just three and
474 two desaturase genes in the same scaffolds of *A. ervi* and *L. fabarum*, respectively).
475 Further investigations should verify this loss in *L. fabarum*, identify the ortholog of the
476 missing copy in *A. ervi*, and test if this potential lost desaturase gene in *L. fabarum* is
477 involved in the generation of unsaturated CHCs in *A. ervi*. This would determine if this
478 loss is a key adaptation for mimicry of their aphid hosts' cuticular hydrocarbon profiles
479 in *L. fabarum*.

480

481 Immune genes

482 We searched for immune genes in the two genomes based on a list of 367 immunity
483 related genes, collected primarily from the *Drosophila* literature (Additional File 8).
484 Using blast-based searches, 204 of these genes (59%) were found and annotated in
485 both species. Six were present in only the *A. ervi* genome and six in only the *L. fabarum*
486 genome. We compared these with the immune genes used to define the main
487 *Drosophila* immune pathways (Toll, Imd, and JAK-STAT, Supplementary Table 10) and
488 conserved in a large number of insect species (Buchon *et al.* 2014; Charroux & Royet
489 2010; Lemaitre & Hoffman 2007). Among these genes there are several well
490 characterized pathways. The *D. melanogaster* Toll pathway is essential for the response
491 to fungi and Gram-positive bacteria (Valanne *et al.* 2011). It was initially identified as a
492 developmental pathway acting via the nuclear factor kappa B (NF- κ B). The Imd/NF-
493 kappa-B pathway is pivotal in the humoral and epithelial immune response to Gram-
494 negative bacteria. Signaling through *imd* (a death domain protein) ultimately activates
495 the transcription of specific antimicrobial peptides (AMPs, Myllymäki *et al.* 2014). The
496 JAK-STAT pathway is involved in the humoral and cellular immune response (Morin-
497 Poulard *et al.* 2013). It is activated after a cytokine-like protein called unpaired (*upd*)
498 binds to its receptor Domeless (Dome). Activated JAK phosphorylates STAT molecules
499 that translocate into the nucleus, where they bind the promoters of target genes.

500 In the genome of both wasps, many genes encoding proteins of the Imd and
501 Toll pathways were absent, such as upstream GNBPs (Gram Negative Binding Proteins)
502 and PGRPs (Peptidoglycan Recognition Proteins) and downstream AMPs
503 (Supplementary Table 10, Supplementary Figure 21, Additional File 8). While none of
504 these genes were found in *L. fabarum*, one PGRP related to PGRP-SD, involved in the

505 response to Gram-positive bacteria (Bischoff *et al.* 2004), and one *defensin*-related
506 gene were found in *A. ervi*. The *imd* gene was also absent in in both wasps; this is
507 noteworthy because *imd* has been present in other hymenopteran genomes analyzed
508 to date. Strikingly, all of the Imd pathway genes, including GGBP- and PGRP-encoding
509 genes, *imd*, *FADD*, *Dredd* and *Relish* are lacking in aphid genomes (*A. pisum*, *A. gossypii*
510 and *D. noxia*, via AphidBase (Legeai *et al.* 2010) and Gerardo *et al.* (2010)), and *imd* is
511 absent in *A. glycines*, *M. persicae*, *M. cerisae*, *R. padi* genomes, some of which are hosts
512 for *A. ervi* and *L. fabarum* (Kavallieratos *et al.* 2004). The lack of an Imd pathway in
513 aphids is suggested to be an adaptation to tolerate the obligate bacterial symbiont,
514 *Buchnera aphidicola*, as well as their facultative endosymbionts that are gram-negative
515 gamma-proteobacteria (e.g. *Hamiltonella defensa*). These facultative symbionts exhibit
516 defensive activities against microbial pathogens and insect parasitoids (Guo *et al.* 2017;
517 Leclair *et al.* 2016; Oliver *et al.* 2010; Scarborough *et al.* 2005) and may at least partially
518 compensate for the host aphids innate immune functions. Recent data also suggest
519 that cross-talk occurs between the Imd and Toll pathways to target wider and
520 overlapping arrays of microbes (Nishide *et al.* 2019). Whether a similar cross-talk occurs
521 in these two Aphidiidae (*A. ervi* and *L. fabarum*) needs further study.

522 Overall, our results suggest convergent evolution of loss in immunity genes, and
523 possibly function, between these parasitoids and their aphid hosts. One reason might
524 be that during the early stages of development, parasitoids need host symbionts to
525 supply their basic nutrients, and thus an immune response from the parasitoid larvae
526 might impair this function. Alternatively, but not exclusively, mounting an immune
527 response against bacteria by the parasitoid larvae may be energetically costly and
528 divert resources from its development. This idea of energy conservation would be

529 especially relevant if the GC-loss discussed above is a mechanism to conserve
530 resources. In both cases, the immune response will be costly for the parasitoid. Further
531 work is needed to address whether other unrelated aphid parasitoids are lacking *imd*,
532 upstream activators, and downstream effectors of the immune pathways (a
533 preliminary blast search suggests that *imd* is present in the Aphelinidae *Aphelinus*
534 *abdominalis*). This impaired immunity might lead to a decrease in both wasps'
535 responses to pathogenic bacteria, or they may use other defensive components to fight
536 bacterial infections (perhaps some in common with aphids) that await to be discovered.
537 For example, in *L. fabarum*, recent transcriptomic work has shown that detoxifying
538 genes may be a key component of parasitoid success (Dennis *et al.* in revision), and
539 these could play a role in immunity.

540

541 **Osiris genes**

542 The Osiris genes are an insect-specific gene family that underwent multiple tandem
543 duplications early in insect evolution. These genes are essential for proper
544 embryogenesis (Smoyer *et al.* 2003) and pupation (Andrade López *et al.* 2017; Schmitt-
545 Engel *et al.* 2015), and are also tied to immune and toxin-related responses (e.g.
546 Andrade López *et al.* 2017; Greenwood *et al.* 2017) and developmental polyphenism
547 (Smith *et al.* 2018; Vilcinskas & Vogel 2016).

548 We found 21 and 25 putative Osiris genes in the *A. ervi* and *L. fabarum*
549 genomes, respectively (Supplementary Tables 11, 12). In insects with well assembled
550 genomes, there is a consistent synteny of approximately 20 Osiris genes; this cluster
551 usually occurs in a ~150kbp stretch and gene synteny is conserved in all known
552 Hymenoptera genomes (Supplementary Figure 22). The Osiris cluster is largely devoid

553 of non-Osiris genes in most of the Hymenoptera, but the assemblies of *A. ervi* and *L.*
554 *fabarum* suggest that if the cluster is actually syntenic in these species, there are
555 interspersed non-Osiris genes (those are black boxes in Supplementary Figures 23 and
556 24).

557 In support of their role in defense (especially metabolism of xenobiotics and
558 immunity), these genes were much more highly expressed in larvae than in adults
559 (Supplementary Table 12). We hypothesize that their upregulation in larvae is an
560 adaptive response to living within a host. Because of the available transcriptomic data,
561 we could only make this comparison in *L. fabarum*. Here, 19 of the 26 annotated Osiris
562 genes were significantly differentially expressed in larvae over adults (Supplementary
563 Table 12, Additional File 9). In both species, transcription in adults was very low, with
564 fewer than 10 raw reads per cDNA library sequenced, and most often less than one
565 read per library.

566

567 OXPPOS

568 In most eukaryotes, mitochondria provide the majority of cellular energy (in the form
569 of adenosine triphosphate, ATP) through the oxidative phosphorylation (OXPPOS)
570 pathway. OXPPOS genes are an essential component of energy production, and have
571 increased in Hymenoptera relative to other insect orders (Li *et al.* 2017). We identified
572 69 out of 71 core OXPPOS genes in both genomes, and identified five putative
573 duplication events that are apparently not assembly errors (Supplementary Table 13,
574 Additional File 10). The gene sets of *A. ervi* and *L. fabarum* contained the same genes
575 and the same genes were duplicated in each, implying duplication events occurred
576 prior to the split from their most recent common ancestor. One of these duplicated

577 genes appears to be duplicated again in *A. ervi*, or the other copy has been lost in *L.*
578 *fabarum*.

579

580 **Chemosensory genes**

581 Genes underlying chemosensory reception play important roles in parasitoid mate and
582 host localization (Comeault *et al.* 2017; Nouhaud *et al.* 2018). Several classes of
583 chemosensory genes were annotated separately (Table 4): odorant receptors (ORs) are
584 known to detect volatile molecules, odorant-binding proteins (OBPs) and
585 chemosensory proteins (CSPs) are possible carriers of chemical molecules to sensory
586 neurons, and ionotropic receptors (IRs) are involved in both odorant and gustatory
587 molecule reception. With these manual annotations, further studies can now be made
588 with respect to life history characters including reproductive mode, specialization on
589 aphid hosts, and mimicry.

590

591 Chemosensory: Soluble proteins (OBPs and CSPs)

592 Hymenoptera have a wide range of known OBP genes, with up to 90 in *N. vitripennis*
593 (Vieira *et al.* 2012). However, the numbers of these genes appear to be similar across
594 parasitic wasps, with 14 in both species studied here and 15 recently described in *D.*
595 *alloeum* (Tvedte *et al.* 2019). Similarly, CSP numbers are in the same range within
596 parasitic wasps (11 and 13 copies here, Table 4). Interestingly, two CSP sequences (one
597 in *A. ervi* and one in *L. fabarum*) did not have the conserved cysteine motif,
598 characteristic of this gene family. So although they were annotated here, further work
599 should investigate if and how these genes function.

600

601 Chemosensory: Odorant receptors (ORs)

602 In total, we annotated 228 putative ORs in *A. ervi* and 156 in *L. fabarum* (Table 4). This
603 is within the range of OR numbers annotated in other hymenopteran parasitoids,
604 including: 79 in *M. cingulum* (Ahmed *et al.* 2016), 225 in *N. vitripennis* (Robertson *et*
605 *al.* 2010), and 187 in *D. alloeum* (Tvedte *et al.* 2019). Interestingly, we annotated a
606 larger set of ORs in *A. ervi* than in *L. fabarum*. One explanation is that *A. ervi* generally
607 has more annotated genes than *L. fabarum*, and whatever broad pattern underlies
608 the reduction in the gene repertoire of *L. fabarum* also affected OR genes. One
609 functional explanations for a lower number of OR genes in *L. fabarum* is that the *A.*
610 *ervi* strain sequenced of was derived from several field strains that parasitized
611 different hosts on different host plants, and the ability to parasitize a broader host
612 range could select for more OR genes (Monticelli *et al.* 2019).

613

614 Chemosensory: Ionotropic chemosensory receptors (IRs)

615 In total, we annotated 38 putative IRs in *A. ervi* and 37 in *L. fabarum* (Table 4). Three
616 putative co-receptors (IR 8a, IR 25a and IR 76b) were annotated both species, one of
617 which (IR 76b) was duplicated in *A. ervi*. This bring the total for the IR functional group
618 to 42 and 40 genes for *A. ervi* and *L. fabarum*, respectively. This is within the range of
619 IRs known from other parasitoid wasps such as *Aphidius gifuensis* (23 IRs identified in
620 antennal transcriptome, Braconidae, Kang *et al.* 2017), *D. alloeum* (51 IRs, Braconidae,
621 Tvedte *et al.* 2019) and *N. vitripennis* (47 IRs, Pteromalidae, Robertson *et al.* 2010). A
622 phylogenetic analysis of these genes showed a deeply rooted expansion in the IR genes
623 (Supplementary Figure 25). Thus, in contrast to the expansion usually observed in
624 hymenopteran ORs compared to other insect orders, IRs have not undergone major

625 expansions in parasitic wasps, which is generally the case for a majority of insects with
626 the exception of Blattodea (Harrison *et al.* 2018)

627

628 **Sex determination**

629 The core sex determination genes (*transformer*, *doublesex*) are conserved in both
630 species (Supplementary Table 14, Additional File 11). Notably, *A. ervi* possesses a
631 putative *transformer* duplication. This scaffold carrying the duplication (scaffold2824)
632 is only fragmentary, but a *transformer* duplicate has also been detected in the
633 transcriptome of a member of the *A. colemani* species complex, suggesting a conserved
634 presence within the genus (Peters *et al.* 2017). In *A. ervi*, *transformer* appears to have
635 an internal repeat of the CAM-domain, as is seen in the genus *Asobara* (Geuverink *et*
636 *al.* 2018). In contrast, there is no evidence of duplication in sex determination genes in
637 *L. fabarum*. This supports the idea that complementary sex determination (CSD) in
638 sexually reproducing *L. fabarum* populations is based on up-stream cues that differ
639 from those known in other CSD species (Matthey-Doret *et al.* 2019), whereas the CSD
640 locus known from other hymenopterans locus is a paralog of *transformer* (Heimpel &
641 de Boer 2007).

642 In addition to the core sex determination genes, we identified homologs of
643 several genes related to sex determination (Supplementary Table 15). We identified
644 *fruitless* in both genomes, which is associated with sex-specific behavior in taxa
645 including *Drosophila* (Yamamoto 2008). Both genomes also have homologs of *sex-lethal*
646 which is the main determinant of sex in *Drosophila* (Bell *et al.* 1988). *Drosophila* has
647 two homologs of this gene, and the single version in Hymenoptera may have more in
648 common with the non-sex-lethal copy, called *sister-of-sex-lethal*. We identified

649 homologs of the gene *CWC22*, including a duplication in *A. ervi*; this duplication is
650 interesting because a duplicated copy of *CWC22* is the primary signal of sex
651 determination in the house fly *Musca domestica* (Sharma *et al.* 2017). Lastly, there was
652 a duplication of *RBP1* in both genomes. The duplication of *RBP1* is not restricted to
653 these species, nor is the duplications of *CWC22*, which appears sporadically in
654 Braconidae. Together, these annotations add to our growing knowledge of duplications
655 of these genes, and provide possibilities for further examinations of the role of
656 duplications and specialization in association with sex determination.

657

658 DNA Methylation genes

659 DNA methyltransferase genes are thought to be responsible for the generation and
660 maintenance of DNA methylation. In general, DNA methyltransferase 3 (*DNMT3*)
661 introduces *de novo* DNA methylation sites and DNA methyltransferase 1 (*DNMT1*)
662 maintains and is essential for DNA methylation (Jeltsch & Jurkowska 2014; Provataris
663 *et al.* 2018). A third gene, *EEF1AKMT1* (formerly known as *DNMT2*), was once thought
664 to act to methylate DNA but is now understood to methylate tRNA (Provataris *et al.*
665 2018). In both *A. ervi* and *L. fabarum*, we successfully identified homologs *DNMT3* and
666 *EEF1AKMT1*. In contrast, *DNMT1* was not detected in either species (Table 4,
667 Supplementary Table 16). This adds to growing evidence that these genes are not
668 conserved across family Braconidae, as *DNMT1* appears to be absent in several other
669 braconid genera, including *Asobara tabida*, *A. japonica*, *Cotesia sp.*, and *F. arisanus*
670 (Bewick *et al.* 2017; Geuverink 2017). However, *DNMT1* is present in some braconids,
671 including *M. demolitor*, and outside of Braconidae these genes are otherwise strongly

672 conserved across insects. In contrast, DNMT3, present here, is more often lost in
673 insects (Provataris *et al.* 2018).

674 This absence of *DNMT1* helps explain previous estimates of very low DNA
675 methylation in *A. ervi* (0.5%, Bewick *et al.* 2017). We confirmed these low levels of
676 methylation in *A. ervi* by mapping this previously generated bisulfite sequencing data
677 (Bewick *et al.* 2017) to our genome assembly. We aligned >80% of their data (total
678 94.5Mbp, 625,765 reads). The sequence coverage of this mapped data was low: only
679 63,554 methylation-available cytosines were covered and only 1,216 were represented
680 by two or more mapped reads. Nonetheless, of these mapped cytosines, the vast
681 majority (63,409) were never methylated, just 143 sites were always methylated, and
682 two were variably methylated. Methylation-available cytosine classes were roughly
683 equally distributed among three cytosine classes (CG: 0.154%, CHG: 0.179%, and CHH:
684 0.201%). This methylation rate is less than the 0.5% estimated by Bewick (2017) and
685 confirms a near absence of DNA methylation in *A. ervi*. Given the parallel absence of
686 DNMT1 in *L. fabarum*, it seems likely that both species sequenced here may have very
687 low levels of DNA methylation, and that this is not a significant mechanism in these
688 species.

689 This stark reduction in DNA methylation is interesting, given that epigenetic
690 mechanisms are likely important to insect defenses, including possible responses to
691 host endosymbionts (Huang *et al.* 2019; Vilcinskas 2016, 2017). As with the immune
692 pathways discussed above, this could reflect a loss that is adaptive to developing within
693 endosymbiont-protected hosts. It is also interesting that while one epigenetic
694 mechanism seems to be absent in both *A. ervi* and *L. fabarum*, we see an increase in
695 histone variants in *L. fabarum* (based on the OMA analysis of gene family expansion),

696 and these histones could function in gene regulation. However, whether there is a
697 functional or causal link between these two observations is yet to be tested.

698
699

Table 4: Summary of annotation of putative DNA methylation genes

Species	Gene	Scaffold	e-value (<i>Nasonia</i>)
<i>A. ervi</i>	EEF1AKMT1 homolog	scaffold94	1.00E-66
<i>L. fabarum</i>		tig00000449	5.00E-63
<i>A. ervi</i>	DNA methyltransferase 3	scaffold45	5.00E-138
<i>L. fabarum</i>		tig00002022	9.00E-117
<i>A. ervi</i>	DNA methyltransferase 1	<i>no homolog detected</i>	
<i>L. fabarum</i>		<i>no homolog detected</i>	

700

701

702 Conclusions

703 These two genomes have provided insight into adaptive evolution in parasitoids that
704 infect aphids. Both genomes are extremely GC-poor, and their extreme codon bias
705 provides an excellent system for examining the chemical biases and selective forces
706 that may overshadow molecular evolution in eukaryotes. We have also highlighted
707 several groups of genes that are key to functional evolution across insects, including
708 venom, sex determination, response to bacterial infection (F-box/*LRR* proteins), and
709 near absence of DNA methylation. Moreover, the absence of certain immune genes
710 (e.g. from the Imd and Toll pathways) in these two species is similar to losses in host
711 aphids, and raises intriguing questions related to the effects of aphids' symbiosis on
712 both aphid and parasitoid genomics.

713 Parasitoid wasps provide an excellent model for studying applied and basic
714 biological questions, including host range (specialist vs generalist), reproductive mode
715 (sexual vs asexual), antagonistic coevolution, genome evolution, and epigenetic
716 regulation, to mention just a few. Our new genomic resources will open the way for a

717 broad set of future research, including work to understand host specialization, adaptive
718 changes associated with climate, and the potential loss of diapause in *A. ervi* (Tougeron
719 *et al.* 2019; Tougeron *et al.* 2017). Lastly, the genomes of these two non-social
720 Hymenoptera provide a valuable comparison for understanding processes specific to
721 social insects with complex caste structure, and are a first but essential step to better
722 understand the genetic architecture and evolution of traits that are important for a
723 parasitic life style and their use in biological control.

724

725

726 **Methods**

727 **More complete methods are available in the Supplementary Material*

728 **Insect collection and origin**

729 *Aphidius ervi*

730 *Aphidius ervi* samples used for whole-genome sequencing came from two different,
731 sexually reproducing, isofemale lines established from parasitized aphids (recognizable
732 as mummies) from fields of cereals and legumes in two different geographic zones in
733 Chile: Region de Los Rios (S 39° 51', W 73° 7') and Region del Maule (S 35° 24', W 71°
734 40'). Mummies (parasitized aphids) of *Sitobion avenae* aphids were sampled on wheat
735 (*Triticum aestivum* L.) while mummies of *Acyrtosiphon pisum* aphids were sampled on
736 *Pisum sativum* L. (pea aphid race). Aphid mummies were isolated in petri dishes until
737 adult parasitoids emerged. These two parasitoid lineages were separated in two cages
738 with hosts *ad libitum* and were propagated for approximately 75 generations under
739 controlled conditions as described elsewhere (Ballesteros *et al.* 2017; Sepúlveda *et al.*
740 2016). A further reduction of genetic variation was accomplished by establishing two

741 isofemale *A. ervi* lines, which were maintained as described previously and propagated
742 for approximately 10 generations before adult parasitoids (male and female) were
743 collected live and stored in 1.5 ml centrifuge tubes containing ethanol (95%) at -20°C.

744 *Aphidius ervi* samples used for CHC analysis (below) were purchased from Katz
745 Biotech AG (Baruth, Germany). Species identification was confirmed with COI
746 barcoding following Hebert *et al.* (2003). Wasps sacrificed for CHC analysis were
747 sampled from the first generation reared in the lab on *Acyrtosiphon pisum* strain LL01
748 (Peccoud *et al.* 2009), which were mass-reared on *Vicia faba* cv. *Dreifach Weisse*.

749

750 *Lysiphlebus fabarum*

751 *Lysiphlebus fabarum* samples used for whole-genome sequencing came from a single,
752 asexually reproducing, isofemale line (IL-07-64). This lineage was first collected in
753 September 2007 from Wildberg, Zürich, Switzerland as mummies of the aphid *Aphis*
754 *fabae fabae*, collected from the host plant *Chenopodium album*. In the lab, parasitoids
755 were reared on *A. f. fabae* raised on broad bean plants (*Vicia faba*) under controlled
756 conditions [16 h light: 8 h dark, 20°C] until sampling in September 2013, or
757 approximately 150 generations. Every lab generation was founded by ca. 10 individuals
758 that were transferred to fresh host plants containing wasp-naïve aphids. Approximately
759 700 individuals were collected for whole-genome sequencing from a single generation
760 in December 2013 and flash frozen at -80°C. To avoid sequencing non-wasp DNA,
761 samples were sorted over dry ice to remove any contaminating host aphid or plant
762 material.

763 For linkage group construction, separate *L. fabarum* collections were made
764 from a sexually reproducing lineage. Here, we collected all sons produced by a single

765 virgin female, sampled from the control lineage in a recently employed evolution
766 experiment (H-lineage; Dennis *et al.* 2017). Wasps were stored on ethanol until RAD-
767 seq library construction. Lastly, a third population was sampled for the proteomic
768 analysis of the venom-apparatus (below); these females came from the genetically-
769 diverse starting population used to found the evolution experiment of Dennis *et al.*
770 (2017), and were sampled in December 2014.

771

772 DNA extraction and library preparation

773 *Aphidius ervi*

774 DNA was extracted from adult haploid males of *A. ervi* in seven sub-samples (ca. 120
775 males each), reared in *S. avenae*. Total DNA was extracted using the DNEasy Plant Mini
776 Kit (QIAGEN) following the manufacturer's instructions. DNA was quantified by
777 spectrophotometry (Epoch Microplate Spectrophotometer, Biotek) and fluorometry
778 (Qubit 3.0; Qubit DNA High sensitivity Assay Kit, Invitrogen), and quality was assessed
779 using 1% agarose gel electrophoresis. DNA samples were sent on dry ice to MACROGEN
780 (Seoul, South Korea) and were used to produce Illumina paired-end (PE) and mate-pair
781 (MP) libraries for sequencing. A PE library was constructed from one of the seven sub-
782 samples (120 individuals, 1 μ g DNA) sheared by ultrasonication (Covaris) company,
783 average sheared insert size: 350bp). The remaining DNA samples were pooled (6
784 samples, 720 individuals) and used for MP sequencing (3kb, 5kb and 8kb insert sizes),
785 which were prepared with the Nextera mate-pair protocol (Illumina). All libraries were
786 sequenced using an Illumina HiSeq 2000 sequencer (MACROGEN).

787 Long read PacBio (Pacific Biosciences) RS II sequencing was performed from a
788 single DNA extraction of 270 *A. ervi* females, reared on *A. pisum*. Genomic DNA was

789 extracted using the Wizard genomic DNA purification kit (Promega) according to
790 manufacturer instructions and quantified spectrophotometrically using a NanoDrop
791 2000 (Thermo Scientific). Input DNA was mechanically sheared to an average size
792 distribution of 10Kb (Covaris gTube, Kbiosciences) and the resulting library was size
793 selected on a Blue Pippin Size Selection System (Cat #BLU0001, Sage Science) to enrich
794 fragments > 8Kb. Quality and quantity were checked on Bioanalyzer (Agilent
795 Technologies) and Qubit, respectively. Four SMRT RSII cells with P6 chemistry were
796 sequenced at GenoScreen, France.

797

798 *Lysiphlebus fabarum*

799 DNA was extracted from adult female *L. fabarum* in 10 sub-samples (50-100 wasps
800 each) using the QIAmp DNA mini Kit (Qiagen) according to the manufacturer's
801 instructions, with the inclusion of an overnight tissue digestion at 56 °C. Extracted DNA
802 was then pooled and used to produce Illumina PE and MP, and PacBio libraries. The PE
803 library was prepared using the Illumina Paired-End DNA protocol; the average fragment
804 size was 180 base pair (bp). The MP library (5kb insert) was generated with the Nextera
805 mate-pair protocol (Illumina). Both libraries were sequenced on the Illumina MiSeq in
806 Paired-End mode at the University of Zürich.

807 Long-read libraries for PacBio RS II sequencing were produced using the DNA
808 Template Prep Kit 2.0 (Pacific Biosciences). Input DNA was mechanically sheared to an
809 average size distribution of 10Kb (Covaris gTube, Kbiosciences) and the resulting library
810 was size selected on a Blue Pippin Size Selection System (Sage Science) machine to
811 enrich fragments > 8Kb; quality and quantity were checked on the Bioanalyzer and
812 Qubit, respectively. Ten SMRT Cells were sequenced at the University of Zürich.

813

814 **Genome assembly**

815 *Aphidius ervi*

816 Library quality was checked with FastQC ver. 0.11.3 (Andrews *et al.* 2010). Paired-end
817 libraries were processed with Trimmomatic ver. 0.35 (Bolger *et al.*, 2014) for trimming
818 Illumina adapters/primers, low quality bases (Q <25, 4bp window) and discarding
819 sequences shorter than 50bp or without its mate-pair. In the case of Mate-Pair libraries,
820 removal of improperly oriented read-pairs and removal of Nextera adapters was
821 performed using NextClip (Leggett *et al.* 2014). Filtered PE and MP libraries were used
822 for genome assembly with Platanus ver. 1.2.1 with default parameters (Kajitani *et al.*
823 2014), gap closing was performed with GapCloser (Luo *et al.* 2012). Scaffolding with
824 PacBio reads was performed using a modified version of SSPACE-LR v1.1 (Boetzer &
825 Pirovano 2014), with the maximum link option set by `-a 250`. Finally, the gaps of this
826 last version were filled with the Illumina reads using GapCloser.

827

828 *Lysiphlebus fabarum*

829 Library quality was also checked with FastQC (Andrews *et al.* 2010). Illumina reads were
830 filtered using Trimmomatic to remove low quality sequences (Q<25, 4bp window), to
831 trim all Illumina primers, and to discard any sequence shorter than 50bp or without its
832 mate-pair. NextClip was used to remove all improperly oriented read pairs.

833 Raw PacBio reads were error-corrected using the quality filtered Illumina data
834 with the program Proovread (Hackl *et al.* 2014). These error-corrected reads were then
835 used for *de novo* assembly in the program *canu* v1.0 (Koren *et al.* 2017). Since our
836 PacBio reads were expected to have approximately 30X coverage (based on the

837 presumed size of 128MB), *Canu* was run with the recommended settings for low
838 coverage data (corMhapSensitivity=high corMinCoverage=2 errorRate=0.035), and
839 with the specification that the genome is approximately 128Mbp. The resulting
840 assembly was polished using Pilon (Walker *et al.* 2014) to correct for both single
841 nucleotide and small indel errors, using mapping of both the MP and PE data,
842 generated with bwa-mem (Li & Durbin 2009).

843

844 Linkage map construction: *L. fabarum*

845 For linkage map construction, we followed the methodology described in Wang *et al.*
846 (2013) and Purcell *et al.* (2014). In brief, we genotyped 124 haploid male offspring from
847 one sexual female using ddRADseq. Whole-body DNA was high-salt extracted (Aljanabi
848 & Martinez 1997), digested with the *EcoRI* and *MseI* restriction enzymes, and ligated
849 with individual barcodes (Parchman *et al.* 2012; Peterson *et al.* 2012). Barcoded
850 samples were purified and amplified with Illumina indexed primers by PCR (Peterson *et*
851 *al.* 2012) and quality-checked on an agarose gel.

852 Pooled samples were sequenced on the Illumina HiSeq2500. Raw single-end
853 libraries were quality filtered and de-multiplexed using the process_radtags routine
854 within Stacks v1.28 with default parameters (Catchen *et al.* 2011), and further filtered
855 for possible adapter contamination using custom scripts. Genotyping was performed by
856 mapping all samples against the *L. fabarum* draft genome assembly using bowtie2
857 (Langmead & Salzberg 2012) with rg-id, sensitive and end-to-end options. Genotypes
858 were extracted using samtools mpileup (Li *et al.* 2009) and bcftools (haploid option, Li
859 2011). We filtered the resulting genotypes for a quality score >20 and removed loci
860 with >20% missing data and/or a minor allele frequency <15% using VCFtools v0.1.12b

861 (Danecek *et al.* 2011). After filtering, 1,319 biallelic SNPs in 90 offspring remained.

862 For constructing linkage groups, we followed Gadau (2009) to account for the
863 unknown phase of the maternal genotype. In short, we duplicated the haploid male
864 genotypes and reversed the phase for one duplicated set and removed one of the
865 mirror linkage group sets after mapping. We generated the map using MSTmap (Wu *et al.*
866 *al.* 2008) on the data with following parameters: population_type DH,
867 distance_function kosambi, no_map_dist 15.0, no_map_size 2, missing_threshold 1.00,
868 and the cut_off_p_value 1e-6. The cut-off p-value was adjusted to create a linkage map
869 of five linkage groups, however the biggest group had a gap of >70 cM, indicating a false
870 fusion of two groups, which we split in two groups. This result corresponded to the six
871 chromosomes previously described for *L. fabarum* (Belshaw & Quicke 2003), these
872 were visualized with AllMaps (Tang *et al.* 2015). Initial mapping showed that 14 SNPs at
873 one end of tig00000000 mapped to Chromosome1, while the majority of the contig
874 (>150,000 bp) mapped to Chromosome 2. Thus, these SNPs were removed from the
875 linkage maps, and it is advised that subsequent drafts of the *L. fabarum* genome should
876 split this contig around position 153,900.

877

878 **Genome completeness and synteny**

879 Completeness of the two assemblies was assessed by identifying Benchmarking
880 Universal Single-Copy Orthologs (BUSCOs) using the BUSCO v3.0.2 pipeline in genome
881 mode (Simão *et al.* 2015). We identified single copy orthologs based on the
882 Arthropoda_db9 (1,066 genes, training species: *Nasonia vitripennis*).

883 Synteny between the two genomes was assessed using the NUCmer aligner,
884 which is part of the MUMmer v3.23 package (Kurtz *et al.* 2004). For this, we used the

885 *L. fabarum* chromosomes as the reference, and included the scaffolds not incorporated
886 into chromosomes (total 1,407 pieces). The *A. ervi* assembly was mapped to this using
887 the default settings of NUCmer.

888

889 Predictive gene annotation

890 For both assembled genomes, gene predictions were generated using MAKER2 (Holt &
891 Yandell 2011). Within MAKER2, predictive training was performed in a three step
892 process. A first set of genes was predicted by similarity to known proteins or contigs
893 from RNAseq in the same species (described below). This gene set was used thereafter
894 for training both Augustus (Keller *et al.* 2011) and SNAP (Korf 2004), in two steps, with
895 the results of the first training re-used to train the software in the second round.
896 Transcriptomic evidence was provided separately for each species. For *A. ervi*, six
897 separate *de novo* transcriptome assemblies from Trinity (Grabherr *et al.* 2011) were
898 constructed, one each for the adults reared on different hosts (NCBI PRJNA377544,
899 Ballesteros *et al.* 2017). For each transcript, we only included variants based on filtering
900 with RSEM v 1.2.21 using the option `-fpm_cutoff 1.0, --isopct_cutoff=15.00`. This
901 resulted in 452,783 transcripts. For *L. fabarum*, we utilized a joint transcriptome, built
902 using RNAseq data (NCBI PRJNA290156) collected from adults (Dennis *et al.* 2017) and
903 4-5 day old larvae (Dennis *et al.* in review). Peptide evidence came from the
904 Hymenoptera genomes database (<http://hymenopteragenome.org>, *Acromyrmex*
905 *echinator* v3.8, *Apis mellifera* v3.2, *Nasonia vitripennis* v1.2), from the Bioinformatics
906 Platform of Agroecosystems Arthropod database (<https://bipaa.genouest.org>,
907 *Hyposoter didymator* v1.0), and *Drosophila melanogaster* (<http://flybase.org>, v6.13), and
908 SwissProt (October 2016) databases. Summary statistics were generated with GAG

909 (Hall *et al.* 2014). Transcriptomic support for the predicted genes was estimated by
910 mapping available transcriptomic data (same as above) to the respective genomes
911 using STAR (Dobin *et al.* 2013) in the “quantMode”.

912

913 **Functional annotation**

914 The putative functions of the proteins predicted by the above pipeline were identified
915 based on blastp (v2.5.0) matches against Genbank *nr* (non-redundant GenBank CDS
916 translations+PDB+SwissProt+PIR+PRF) release 12/2016 and interproscan v5 against
917 Interpro (1.21.2017). GO terms associations were collected from blast *nr* and
918 interproscan results with blast2GO (v2.2). Finally, transmembrane domains were
919 identified with Hidden Markov Models (HMM) in tmhmm v2.0c, and peptide signals
920 with signalP (euk v4.1, Emanuelsson *et al.* 2007; Nielsen 2017).

921

922 **Transposable elements**

923 Transposable elements (TE) were predicted using the REPET pipeline (Flutre *et al.*
924 2011), combining *de novo* and homology-based annotations. *De novo* prediction of TEs
925 was restricted to scaffolds larger than the scaffold N50 for each species. Within these,
926 repetitive elements were identified using a blast-based alignment of each genome to
927 itself followed by clustering with Recon (Bao & Eddy 2002), Grouper (Quesneville *et al.*
928 2005) and Piler (Edgar & Myers 2005). For each cluster, a consensus sequence was
929 generated by multiple alignment of all clustered elements with MAP (Huang 1994). The
930 resulting consensus was then scanned for conserved structural features or homology
931 to nucleotide and amino acid sequences from known TEs (RepBase 20.05, Bao *et al.*
932 2015; Jurka 1998) using BLASTER (tblastx, blastx, Flutre *et al.* 2011) or HMM profiles of

933 repetitive elements (Pfam database 27.0) using hmmer3 (Mistry *et al.* 2013). Based on
934 identified features, repeats were classified using Wicker's TE classification as
935 implemented in the PASTEclassifier (Hoede *et al.* 2014). The resulting *de novo* TE library
936 for the genome was then filtered to retain only the elements with at least one perfect
937 match in the genome. Subsequently, all TEs in the genomes were annotated with
938 REPET's TE annotation pipeline. Reference TE sequences were aligned to the genome
939 using BLASTER, Repeat Masker (Smit *et al.* 2013-2015) and CENSOR (Kohany *et al.*
940 2006). The resulting HSPs were filtered using an empirical statistical filter implemented
941 in REPET (Flutre *et al.* 2011) and combined using MATCHER (Quesneville *et al.* 2005).
942 Short repeats were identified using TRF (Benson 1999) and Mreps (Kolpakov *et al.*
943 2003). Elements in genomic sequences with homology with known rebase elements
944 (RepBase 20.05) were identified with BLASTER (blastx, tblastx) and curated by
945 MATCHER. Finally, redundant TEs and spurious SSR annotations were filtered and
946 separate annotations for the same TE locus were combined using REPET's "long join
947 procedure".

948

949 **GC content and codon usage**

950 We examined several measures of nucleotide composition, at both the nucleotide and
951 protein level. Whole genome GC content was calculated by totaling the numbers of A,
952 C, T, and G in the entire assembly. In the predicted coding sequences, this was also
953 calculated separately for each predicted gene and third position GC composition was
954 calculated separately in the predicted coding sequences. In all cases, this was done with
955 the sscu package in R (Sun 2016). Relative Synonymous Codon Usage (RSCU) was
956 extracted from the entire CDS using the seqinR package in R (Charif & Lobry 2007), and

957 visualized with a PCA (R packages factoextra, reshape, and ggplot2, Kassambara &
958 Mundt 2016; Wickham 2007, 2009). To examine GC content in coding genes of other
959 insects, we downloaded the 118 available CDS in the RefSeq database of NCBI (date:
960 October 2018) and again calculated per-gene GC content.

961 To examine the GC content of life-stage biased transcripts, we compared GC
962 content in the genes that are significantly (FDR < 0.05) differentially expressed between
963 previously generated transcriptomes from adult (Dennis *et al.* 2017) and larval (Dennis
964 *et al.* in revision) *L. fabarum*, as well in the 10% most highly expressed genes in adults
965 and larvae.

966

967 **Orphan genes**

968 We identified orphan genes as those for which we could not find orthologs in any other
969 sequenced genomes. To do this, we first used OrthoFinder (Emms & Kelly 2015) to
970 generate clusters of orthologous and paralogous genes among the predicted genes
971 (CDS) from the genomes of *A. ervi* and *L. fabarum*, as well as five other sequenced
972 parasitoids (*Diachasma alloeum*, *Fopius arisanus*, *Macrocentrus cingulum*, *Microplitis*
973 *demolitor* and *Nasonia vitripennis*). OrthoFinder produces a set of genes that were not
974 assigned to any orthogroup. We identified species specific genes, which we are calling
975 orphan genes, by removing all genes that had hits to any other genes in the *nt*, *nr*, and
976 *swissprot* NCBI database (June 2019). Within these putative orphans, we only retained
977 those with transcriptomic support.

978

979

980

981 **Gene family expansions**

982 We examined gene families that have expanded and contracted in *A. ervi* and *L.*
983 *fabarum* relative to one another using the OMA standalone package (v2.2.0, default
984 values, Altenhoff *et al.* 2018). OMA was used to compute orthologs (OMA groups) and
985 Hierarchical Orthologous Groups (HOGs) for the predicted proteins of *L. fabarum* and
986 *A. ervi*: 15,203 and 20,344, respectively. While OMA groups consist of strict 1:1
987 orthologs between OGS1 and OGS3, HOGs contain all orthologs and paralogs of a given
988 predicted gene family. HOGs were parsed with a custom Perl script to identify all gene
989 families in which one of the wasp species contained more members than the other. We
990 focused on only the groups that contained more than 20 genes (ten groups,
991 Supplementary Figure 12). These were identified by blastx against the *nr* database in
992 NCBI.

993

994 **Venom proteins**

995 The *L. fabarum* venom proteomic analysis was performed from 10 extracted venom
996 glands (Supplementary Figure 14). The 16 most visible bands in 1D gel electrophoresis
997 were cut, digested with trypsin and analyzed by mass spectrometry. All raw data files
998 generated by mass spectrometry were processed to generate mgf files and searched
999 against: (i) the *L. fabarum* proteome predicted from the genome (*L. fabarum*
1000 annotation v1.0 proteins) and (ii) the *L. fabarum de novo* transcriptome (Dennis *et al.*
1001 2017) using the MASCOT software v2.3 (Perkins *et al.* 1999). The mass spectrometry
1002 proteomics data have been deposited to the ProteomeXchange Consortium
1003 (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner repository
1004 (Hanrahan & Johnston 2011), with the ID PXD015758.

1005 Sequence annotation was performed based on blast similarity searches. Signal
1006 peptide prediction was performed with SignalP (Emanuelsson *et al.* 2007; Nielsen
1007 2017). Searches for protein domains was performed with PfamScan (Finn *et al.* 2013)
1008 and venom protein genes were identified using the blast tools in Apollo (Dunn *et al.*
1009 2019; Lee *et al.* 2013). Multiple amino acid sequence alignments were made with
1010 MUSCLE (Edgar 2004a, b). Phylogenetic analysis was performed using maximum
1011 likelihood (ML) with PhyML 3.0 (Guindon *et al.* 2010). SMS was used to select the best-
1012 fit model of amino acid substitution for ML phylogeny (Lefort *et al.* 2017).

1013

1014 **Manual gene curation**

1015 The two genome assemblies were manually curated for a number of gene families of
1016 interest. This improved their structural and functional annotation for more in-depth
1017 analysis. Manual curation, performed in Apollo included the inspection of stop/start
1018 codons, duplications (both true and erroneous), transcriptomic support, and
1019 concordance with the predicted gene models.

1020

1021 ***Desaturases***

1022 Desaturase genes in both genomes were automatically identified and annotated with
1023 GeMoMa (Keilwagen *et al.* 2016) using desaturase gene annotations from *Diachasma*
1024 *alloeum*, *Fopius arisanus*, and *Microplitis demolitor*, retrieved from NCBI's protein
1025 database as queries (retrieved May 2017). Additionally, all desaturase genes were
1026 manually inspected.

1027 To measure the production of desaturases in *A. ervi*, wasps were freeze-killed
1028 and stored separately by sex at - 20 °C. For CHC extraction, single individuals were

1029 covered with 50 μ l of MS pure hexane (UniSolv) in 2 ml GC vials (Agilent Technologies),
1030 and swirled for 10 minutes on a Thermo-shaker (IKA KS 130 Basic, Staufen). The hexane
1031 extracts were then transferred to a fresh conical 250 μ l GC insert (Agilent
1032 Technologies), where the hexane was completely evaporated under a constant flow of
1033 CO₂. The dried extract was then resuspended in 5 μ l of a hexane solution containing
1034 7.5 ng/ μ l of n-dodecane (EMD Millipore Corp.) as an internal standard. 3 μ l of the
1035 extract were then injected into a GC-QQQ Triple Quad (GC: 7890B, Triple Quad: 7010B,
1036 Agilent) with a PAL Autosampler system operating in electron impact ionization mode.
1037 The split/splitless injector was operated at 300 °C in Pulsed splitless mode at 20 psi until
1038 0.75 min with the Purge Flow to Split Vent set at 50 mL/min at 0.9 min. Separation of
1039 compounds was performed on a 30 m x 0.25 mm ID x 0.25 μ m HP-1
1040 Dimethylpolysiloxane column (Agilent) with a temperature program starting from 60
1041 °C, held for 2 min, and increasing by 50 °C per min to 200 °C, held for 1 min, followed
1042 by an increase of 8 °C per min to 250 °C, held again for 1 min, and finally 4 °C per min
1043 to 320 °C, held for 10 min. Post Run was set to 325 °C for 5 min. Helium served as carrier
1044 gas with a constant flow of 1.2 ml per min and a pressure of 10.42 psi. Initially CHC
1045 peaks were identified and the chromatogram was generated using the Qualitative
1046 Analysis Navigator of the MassHunter Workstation Software (vB.08.00 / Build
1047 8.0.8208.0, Agilent). CHC quantification was performed using the Quantitative Analysis
1048 MassHunter Workstation Software (vB.09.00 / Build 9.0.647.0, Agilent). Peaks were
1049 quantified using their diagnostic (or the neighboring most abundant) ion as quantifier
1050 and several characteristic ions in their mass spectra as qualifiers to allow for
1051 unambiguous detection by the quantification software. The pre-defined integrator
1052 Agile 2 was used for the peak integration algorithm to allow for maximum flexibility. All

1053 peaks were then additionally checked for correct integration and quantification, and,
1054 where necessary, re-integrated manually. Percentages were based on the respective
1055 averages of four individual female CHC extracts.

1056

1057 **Immune genes**

1058 The list of immune genes to be searched against the *A. ervi* and *L. fabarum* genomes
1059 was established based on *Drosophila melanogaster* lists from the Lemaitre laboratory
1060 (lemaitrelab.epfl.ch/fr/ressources, adapted from De Gregorio *et al.* 2001; De Gregorio
1061 *et al.* 2002) and from the interactive fly web site
1062 (www.sdbonline.org/sites/fly/aigfam/immune.htm and Buchon *et al.* 2014). Each *D.*
1063 *melanogaster* protein sequence was used in blast similarity searches against the two
1064 predicted wasp proteomes. The best match was retained, and its protein sequence was
1065 used to perform a new blast search using the NCBI non-redundant protein sequence
1066 database to confirm the similarity with the *D. melanogaster* sequence. When both
1067 results were concordant, the retained sequence was then searched for in *Nasonia*
1068 *vitripennis* and *Apis mellifera* proteomes to identify homologous genes in these species.

1069

1070 **Osiris genes**

1071 Osiris gene orthologs were determined with a two-part approach: candidate gene
1072 categorization followed by phylogenetic clustering. Candidate Osiris genes were
1073 generated using HMM (with hmmer v3.1b2, Wheeler & Eddy 2013) and local alignment
1074 searching (blast, Altschul *et al.* 1990). A custom HMM was derived using all 24 well
1075 annotated and curated Osiris genes of *Drosophila melanogaster*. Next, an HMM search
1076 was performed on the *A. ervi* and *L. fabarum* proteomes, extracting all protein models

1077 with $P < 0.05$. Similarly, all *D. melanogaster* Osiris orthologs were searched in the
1078 annotated proteomes of *A. ervi* and *L. fabarum* using protein BLAST ($e < 0.05$). The top
1079 BLAST hit for each ortholog was then searched within each parasitoid genome for
1080 additional paralogs ($e < 0.001$). All unique candidates from the above approaches were
1081 then aligned using MAFFT (Kato & Standley 2013), and an approximate maximum-
1082 likelihood phylogeny was constructed using FastTree (Price *et al.* 2009) via the CIPRES
1083 science gateway of Xsede (Miller *et al.* 2015). The species used were: the fruit fly (*D.*
1084 *melanogaster*), the tobacco hornworm moth (*Manduca sexta*), the silkworm moth
1085 (*Bombyx mori*), the flour beetle (*Tribolium castaneum*), the jewel wasp (*Nasonia*
1086 *vitripennis*), the honeybee (*Apis mellifera*), the buff tail bumble bee (*Bombus terrestris*),
1087 the red harvester ant (*Pogonomyrmex barbatus*), the Florida carpenter ant
1088 (*Camponotus floridanus*), and Jerdon's jumping ant (*Harpegnathos saltator*).

1089

1090 OXPPOS

1091 Genes involved in the oxidative phosphorylation pathway (OXPPOS) were identified in
1092 several steps. Initial matches were obtained using the nuclear-encoded OXPPOS
1093 proteins from *Nasonia vitripennis* (Gibson *et al.* 2010; J. D. Gibson unpublished) and
1094 *Drosophila melanogaster* (downloaded from www.mitocomp.uniba.it: Porcelli *et al.*
1095 2007). These two protein sets were used as queries to search the protein models
1096 predicted for *A. ervi* and *L. fabarum* (blastp, Altschul *et al.* 1997). Here, preference was
1097 given to matches to *N. vitripennis*. Next, genes from the *N. vitripennis* and *D.*
1098 *melanogaster* reference set that did not have a match in the predicted proteins were
1099 used as queries to search the genome-assembly (blastn), in case they were not in the
1100 predicted gene models. Gene models for all matches were then built up manually,

1101 based on concurrent evidence from the matches in both *A. ervi* and *L. fabarum* and
1102 their available expression evidence. The resulting protein models were aligned to one
1103 another and to *N. vitripennis* using MAFFT (Katoh & Standley 2013) to identify missing
1104 or extraneous sections. These results were used as queries to search the *N. vitripennis*
1105 proteins to ensure that all matches are reciprocal-best-blast-hits. Gene naming was
1106 assigned based on the existing *N. vitripennis* nomenclature. Potential duplicates were
1107 flagged based on blast-matches back to *N. vitripennis* (Additional Data 10).

1108

1109 **Olfactory genes**

1110 Odorant-binding proteins (OBPs) and chemosensory Proteins (CSPs)

1111 To identify OBPs based on homology to known sequences, we retrieved 60 OBP amino
1112 acid sequences from other Braconidae (namely *Fopius arisanus* and *Microplitis*
1113 *demolitor*) from GenBank. To this, we added seven OBPs found in a previous
1114 transcriptome of *A. ervi* (Patrizia Falabella, unpublished, EBI SRI Accessions:
1115 ERS3933807- ERS3933809). To identify CSPs, we used CSP amino acid sequences from
1116 more Hymenoptera species (*Apis mellifera*, *Nasonia vitripennis*, *Fopius arisanus* and
1117 *Microplitis demolitor*). These sets were used as query against *A. ervi* and *L. fabarum*
1118 genomes using tblastn (e-value cutoff 10e-3 for OBPs and 10e-2 for CSPs). Genomic
1119 scaffolds that presented a hit with at least one of the query sequences were selected.
1120 To identify precise intron/exon boundaries, the Braconidae OBP and CSP amino acid
1121 sequences were then aligned on these scaffolds with Scipio (Keller *et al.* 2008) and
1122 Exonerate (Slater & Birney 2005). These alignments were used to generate gene
1123 models in Apollo. Gene models were manually curated based on homology with other
1124 Hymenoptera OBP and CSP genes and on RNAseq data, when available. Lastly, the

1125 deduced amino acid sequences of *A. ervi* and *L. fabarum* OBP and CSP candidates were
1126 then used as query for another tblastn search against the genomes in an iterative
1127 process to identify any additional OBPs. Since both OBPs and CSPs are secreted
1128 proteins, the occurrence of a signal peptide was verified using SignalP (Emanuelsson *et*
1129 *al.* 2007; Nielsen 2017).

1130

1131 Odorant receptors (ORs)

1132 ORs were annotated using available OR gene models from *Diachasma alloeum*, *Fopius*
1133 *arisanus*, and *Microplitis demolitor* retrieved from NCBI's protein database (retrieved
1134 May 2017). Preliminary OR genes models for *A. ervi* and *L. fabarum* were predicted with
1135 exonerate (v2.4.0), GeMoMa (v1.4, Keilwagen 2016), and combined with Evidence
1136 Modeler (v.1.1.1, Haas *et al.* 2008). These preliminary models were subsequently
1137 screened for the 7tm_6 protein domain (with PfamScan v1.5) and manually curated in
1138 WebApollo2.

1139 In an iterative approach, we annotated the IRs using known IR sequences from
1140 *Apis mellifera*, *Drosophila melanogaster*, *Microplitis demolitor* and *Nasonia vitripennis*
1141 as queries to identify IRs in the genomes of *A. ervi* and *L. fabarum*. The hymenopteran
1142 IR sequences served as input for the prediction of initial gene model with Exonerate
1143 (Slater & Birney 2005) and GeMoMa (Keilwagen *et al.* 2016). Then, we inspected and
1144 edited homologous gene models from each tool in the Apollo genome browser to
1145 adjust for proper splice sites, start and stop codons in agreement with spliced RNA-Seq
1146 reads. After a first round of prediction, we repeated the whole process and provided
1147 the amino acid sequences of curated IR genes as queries for another round of
1148 predictions to identify any remaining paralogous IRs.

1149 Multiple sequence alignments of the IRs were computed with hmalign (Eddy
1150 1998) using a custom IR HMM to guide the alignments (Harrison *et al.* 2018). Gene
1151 trees were generated with FastTree v2 (Price *et al.* 2010) using the pseudocount option
1152 and further parameters for the reconstruction of an exhaustive, accurate tree (options:
1153 -pseudo -spr 4 -mlacc 2 -slowni). Resulting trees were visualized with iTOL v4 (Letunic
1154 & Bork 2019), well supported IR clusters and expansions were highlighted by color
1155 (branch support > 0.9).

1156

1157 ***Sex Determination***

1158 Ortholog searches were performed with tblastn (Altschul *et al.* 1997) against the
1159 genomic scaffolds. Hits with an e-value smaller than 1e-20 were assessed, apart from
1160 *transformer* and *doublesex* where any hit was surveyed. Doublesex, Transformer-2 and
1161 Transformer peptide sequences of *Asobara tabida* (NCBI accessions MF074326-
1162 MF074334) were used as queries for the core sex determination genes. This braconid
1163 species is the closest relative whose sex determination mechanism has been examined
1164 (Geuverink *et al.*, 2018). The putative *transformerB* sequence of *A. ervi* was blasted for
1165 verification against the transcriptome of *Aphidius colemani* (Peters *et al.* 2017) and a
1166 highly conserved fragment was detected (GBVE01021531). Peptide sequences of sex
1167 determination related genes to use as queries were taken from *Nasonia vitripennis*:
1168 Fruitless (NP_001157594), Sex-Lethal homolog (XP_016836645), pre-mRNA-splicing
1169 factor *CWC22* homolog (XP_001601117) and RNA-binding protein 1-like
1170 (XP_008202465). Hidden Markov models were not used as gene models because the
1171 ensuing peptide predictions did not contain all putative homologs (e.g. *transformerB* in
1172 *A. ervi*) due to fragmentation of the scaffolds containing the candidate genes.

1173

1174 ***DNA methylation genes***

1175 The genomes were searched with tblastn (Altschul *et al.* 1997) for the presence of
1176 potential DNA methyltransferase genes using peptide sequences from *Apis mellifera*
1177 and *N. vitripennis* as queries. These species differ in their copy number of *DNMT1*, with
1178 two copies (NP_001164522, XP_006562865) in the honeybee *A. mellifera* (Wang *et al.*
1179 2006) and three copies (NP_001164521, XP_008217946, XP_001607336) in the wasp
1180 *N. vitripennis* (Werren *et al.* 2010). *DNMT2*, currently characterized as EEF1AKMT1
1181 (EEF1A Lysine Methyltransferase 1), has become redundant in the list of DNA
1182 methyltransferase genes as it methylates tRNA instead, but was surveyed here as a
1183 positive control (*N. vitripennis* NP_001123319, *A. mellifera* XP_003251471). *DNMT3*
1184 peptide sequences from *N. vitripennis* (XP_001599223) and from *A. mellifera*
1185 (NP_001177350) were used as queries for this gene. Low levels of methylation were
1186 confirmed by mapping the whole genome bisulfite sequencing data generated by
1187 Bewick *et al.* (2017) back to the *A. ervi* genome assembly.

1188

1189

1190

1191

1192

1193

1194

1195

1196

1197 **List of abbreviations**

1198 **A, T, C, G, and U:** Adenine, Thymine , Cytosine, Guanine, and Uracile, nucleotides

1199 **bp:** Base Pair

1200 **BIPAA:** Bioinformatics Platform for Agroecosystem Arthropods (bipaa.genouest.org)

1201 **BUSCO:** Benchmarking Universal Single-Copy Orthologs

1202 **CDS:** Predicted Coding Sequence

1203 **CSD:** Complementary Sex Determination

1204 **CHC:** Cuticular Hydrocarbons

1205 **DNMT:** DNA Methyltransferase genes

1206 **CSP:** Chemosensory Protein

1207 **GO:** Gene Ontology

1208 **HMM:** Hidden Markov Model

1209 **HOG:** Hierarchical Ortholog Group

1210 **IR:** Ionotropic Receptor

1211 **LRR:** Leucine Rich Repeat Proteins

1212 **Mbp:** Mega Base Pairs, or 1,000,000bp

1213 **MP:** Mate-pair sequence data

1214 **NCBI:** National Center for Biotechnology Information

1215 **N50:** A measure of genome completeness. The length of the scaffold containing the
1216 middle nucleotide

1217 **OXPHOS:** Oxidative Phosphorylation

1218 **OBP:** Odorant-binding Protein

1219 **OR:** Odorant Receptor

1220 **PE:** Paired-end sequence data

1221 **RSCU:** Relative Synonymous Codon Usage

1222 **TE:** Transposable Element

1223

1224

1225

1226

1227

1228

1229

1230

1231

1232

1233

1234

1235

1236

1237

1238

1239

1240

1241

1242

1243 **Availability of data and materials**

1244 Both genomes are available from the NCBI Genome database (PRJNA587428, *A. ervi*:
1245 SAMN13190903, *L. fabarum*: SAMN13190904). The assemblies, predicted genes, and
1246 annotations are also available at <https://bipaa.genouest.org>. Raw Illumina and PacBio
1247 sequence data used to construct genomes is available in NCBI SRA for both *A. ervi*
1248 (SAMN12878248) and *L. fabarum* (accessions SAMN10617865, SAMN10617866,
1249 SAMN10617867), and is further detailed in Supplementary Tables 1 and 2. Venom
1250 protein data are available via ProteomeXchange with identifier PXD015758.

1251

1252

1253

1254

1255

1256

1257 Acknowledgements

1258 Thanks to the many people who helped with collections and insect rearing, especially
1259 Paula Rodriguez (EAWAG), Laury Arthaud and Christian Rebuf (ESIM, INRA-ISA),
1260 Francisca Zepeda-Paulo (UACH), Sebastian Ortiz-Martinez, Cinthya Villegas, and
1261 Daniela Sepúlveda (UTALCA). Lucia Briones (UTALCA) and Dominique Cazes (ESIM,
1262 INRA-ISA) provided help in with *A. ervi* DNA extractions. Paul Saffert (Uni Potsdam)
1263 provided valuable discussion leading to the codon usage analysis. David Pratella (ESIM,
1264 INRA-ISA) provided help on the annotation of immune genes.

1265 *Aphidius ervi* sequencing was funded by FONDECYT grant 1130483 and
1266 Iniciativa Científica Milenio (ICM) NC120027 (both to Christian Figueroa and Blas
1267 Lavandero, Universidad de Talca, Chile), INRA (AIP “séquençage” INRA Rennes, France),
1268 and funding from ESIM team (Marylène Poirié, INRA-ISA Sophia Antipolis, France) and
1269 BGI (funding to Denis Tagu, INRA Rennes, France). The ESIM team is supported by the
1270 French Government (National Research Agency, ANR) through the "Investments for the
1271 Future" LABEX SIGNALIFE : program reference # ANR-11-LABX-0028-01. Mark Lammers
1272 would like to thank Panagiotis Provataris (ZFMK, Bonn, Germany) for bringing to our
1273 attention the existent small whole genome bisulfite sequencing data set for *Aphidius*
1274 *ervi*.

1275 *Lysiphlebus fabarum* data were generated in collaboration with the Genetic
1276 Diversity Centre (GDC, with particular thanks to Stefan Zoller and Jean-Claude Walser),
1277 ETH Zurich, and utilized the ETH Scientific Computing Cluster (Euler). Orthologs were
1278 computed on the University of Potsdam's High Performance Computing Cluster
1279 Orson2, managed by the ZIM. *Lysiphlebus fabarum* sequencing was funded by an SNSF
1280 professorship to Christoph Vorburger (grant nrs. PP00P3_123376 and

1281 PP00P3_146341). TS acknowledges SNSF grant nr PP00P3_170627. JG and LS
1282 acknowledge DFG grant SPP 1819 Rapid evolutionary adaptation (GA 661/4-1, SCHR
1283 1554/3-1).

1284 DNA sequences from the *Myzus* genomes used in comparative analysis of
1285 codon usage were downloaded from AphidBase. Funding for *Myzus persicae* clone
1286 G006 genomic sequencing was provided by USDA-NIFA award 2010-65105-20558.
1287 Funding for *M. persicae* clone O genomic sequencing was provided by The Genome
1288 Analyses Centre (TGAC) Capacity and Capability Challenge program (project CCC-15 and
1289 BB/J004553/1), from the Biotechnology and Biological Sciences Research Council
1290 (BBSRC), and the John Innes Foundation.

1291

1292 **Statement on competing interests**

1293 The authors declare no competing interests

1294

1295

1296

1297

1298

1299

1300

1301

1302

1303

1304

1305

1306 REFERENCES

- 1307 Acquisti C, Elser JJ, Kumar S (2009) Ecological nitrogen limitation shapes the DNA
1308 composition of plant genomes. *Molecular Biology and Evolution* **26**, 953-956.
- 1309 Ahmed T, Zhang T, Wang Z, He K, Bai S (2016) Gene set of chemosensory receptors
1310 in the polyembryonic endoparasitoid *Macrocentrus cingulum*. *Scientific*
1311 *Reports* **6**, 24078-24078.
- 1312 Aljanabi S, Martinez I (1997) Universal and rapid salt-extraction of high quality
1313 genomic DNA for PCR-based techniques. *Nucleic Acids Research* **25**, 4692 -
1314 4693.
- 1315 Almpanis A, Swain M, Gatherer D, McEwan N (2018) Correlation between bacterial
1316 G+C content, genome size and the G+C content of associated plasmids and
1317 bacteriophages. *Microbial Genomics* **4**, -.
- 1318 Altenhoff AM, Levy J, Zarowiecki M, *et al.* (2018) OMA standalone: orthology
1319 inference among public and custom genomes and transcriptomes. *bioRxiv*.
- 1320 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment
1321 search tool. *Journal of Molecular Biology* **215**, 403-410.
- 1322 Altschul SF, Madden TL, Schäffer AA, *et al.* (1997) Gapped BLAST and PSI-
1323 BLAST: a new generation of protein database search programs. *Nucleic Acids*
1324 *Research* **25**, 3389-3402.
- 1325 Andrade López JM, Lanno SM, Auerbach JM, *et al.* (2017) Genetic basis of octanoic
1326 acid resistance in *Drosophila sechellia*: functional analysis of a fine-mapped
1327 region. *Molecular Ecology* **26**, 1148-1160.
- 1328 Andrews S, Krueger F, Segonds-Pichon A, *et al.* (2010) FastQC: a quality control tool
1329 for high throughput sequence data. Available online at:
1330 <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- 1331 Ardila-Garcia AM, Umphrey GJ, Gregory TR (2010) An expansion of the genome
1332 size dataset for the insect order Hymenoptera, with a first test of parasitism
1333 and eusociality as possible constraints. *Insect Molecular Biology* **19**, 337-346.
- 1334 Ballesteros GI, Gadau J, Legeai F, *et al.* (2017) Expression differences in *Aphidius*
1335 *ervi* (Hymenoptera: Braconidae) females reared on different aphid host
1336 species. *PeerJ* **5**, e3640.
- 1337 Bao W, Kojima KK, Kohany O (2015) Repbase Update, a database of repetitive
1338 elements in eukaryotic genomes. *Mobile DNA* **6**, 11.
- 1339 Bao Z, Eddy SR (2002) Automated *de novo* identification of repeat sequence families
1340 in sequenced genomes. *Genome Research* **12**, 1269-1276.
- 1341 Barahimipour R, Strenkert D, Neupert J, *et al.* (2015) Dissecting the contributions of
1342 GC content and codon usage to gene expression in the model alga
1343 *Chlamydomonas reinhardtii*. *The Plant Journal* **84**, 704-717.
- 1344 Behura SK, Severson DW (2013) Codon usage bias: causative factors, quantification
1345 methods and genome-wide patterns: with emphasis on insect genomes.
1346 *Biological Reviews* **88**, 49-61.
- 1347 Bell LR, Maine EM, Schedl P, Cline TW (1988) Sex-lethal, a *Drosophila* sex
1348 determination switch gene, exhibits sex-specific RNA splicing and sequence
1349 similarity to RNA binding proteins. *Cell* **55**, 1037-1046.
- 1350 Belshaw R, Quicke DL (2003) The cytogenetics of thelytoky in a predominantly
1351 asexual parasitoid wasp with covert sex. *Genome* **46**, 170-173.

- 1352 Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences.
1353 *Nucleic Acids Research* **27**, 573-580.
- 1354 Bentele K, Saffert P, Rauscher R, Ignatova Z, Blüthgen N (2013) Efficient translation
1355 initiation dictates codon usage at gene start. *Molecular systems biology* **9**, 675-
1356 675.
- 1357 Bewick AJ, Vogel KJ, Moore AJ, Schmitz RJ (2017) Evolution of DNA methylation
1358 across insects. *Molecular Biology and Evolution* **34**, 654-665.
- 1359 Bischoff V, Vignal C, Boneca IG, *et al.* (2004) Function of the *Drosophila* pattern-
1360 recognition receptor PGRP-SD in the detection of Gram-positive bacteria.
1361 *Nature Immunology* **5**, 1175-1180.
- 1362 Boetzer M, Pirovano W (2014) SSPACE-LongRead: scaffolding bacterial draft
1363 genomes using long read sequence information. *BMC Bioinformatics* **15**, 211.
- 1364 Boivin G, Hance T, Brodeur J (2012) Aphid parasitoids in biological control. *Can J*
1365 *Plant Sci* **92**.
- 1366 Buchon N, Silverman N, Cherry S (2014) Immunity in *Drosophila melanogaster* —
1367 from microbial recognition to whole-organism physiology. *Nature Reviews*
1368 *Immunology* **14**, 796.
- 1369 Burke GR, Strand MR (2014) Systematic analysis of a wasp parasitism arsenal.
1370 *Molecular Ecology* **23**, 890-901.
- 1371 Burke GR, Walden KKO, Whitfield JB, Robertson HM, Strand MR (2018) Whole
1372 genome sequence of the parasitoid wasp *Microplitis demolitor* that harbors an
1373 endogenous virus mutualist. *G3: Genes/Genomes/Genetics*.
- 1374 Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH (2011) Stacks:
1375 Building and genotyping loci *de novo* from short-read sequences. *G3: Genes,*
1376 *Genomes, Genetics* **1**, 171-182.
- 1377 Chaney JL, Clark PL (2015) Roles for synonymous codon usage in protein
1378 biogenesis. *Annual Review of Biophysics* **44**, 143-166.
- 1379 Charif D, Lobry JR (2007) SeqinR 1.0-2: A contributed package to the R project for
1380 statistical computing devoted to biological sequences retrieval and analysis.
1381 In: *Structural Approaches to Sequence Evolution: Molecules, Networks,*
1382 *Populations* (eds. Bastolla U, Porto M, Roman HE, Vendruscolo M), pp. 207-
1383 232. Springer Berlin Heidelberg, Berlin, Heidelberg.
- 1384 Charroux B, Royet J (2010) *Drosophila* immune response: From systemic
1385 antimicrobial peptide production in fat body cells to local defense in the
1386 intestinal tract. *Fly* **4**, 40-47.
- 1387 Chau A, Mackauer M (2000) Host-instar selection in the aphid parasitoid *Monoctonus*
1388 *paulensis* (Hymenoptera: Braconidae, Aphidiinae): a preference for small pea
1389 aphids. *EJE* **97**, 347-353.
- 1390 Chen X-x, van Achterberg C (2018) Systematics, phylogeny, and evolution of
1391 braconid wasps: 30 years of progress. *Annual Review of Entomology*.
- 1392 Cheng R-X, Meng L, Mills NJ, Li B (2011) Host preference between symbiotic and
1393 aposymbiotic *Aphis fabae*, by the aphid parasitoid, *Lysiphlebus ambiguus*.
1394 *Journal of Insect Science* **11**, 81-81.
- 1395 Colinet D, Anselme C, Deleury E, *et al.* (2014) Identification of the main venom
1396 protein components of *Aphidius ervi*, a parasitoid wasp of the aphid model
1397 *Acyrtosiphon pisum*. *BMC Genomics* **15**, 342.
- 1398 Comeault AA, Serrato-Capuchina A, Turissini DA, *et al.* (2017) A nonrandom subset
1399 of olfactory genes is associated with host preference in the fruit fly *Drosophila*
1400 *orena*. *Evolution Letters* **1**, 73-85.

- 1401 Danecek P, Auton A, Abecasis G, *et al.* (2011) The variant call format and VCFtools.
1402 *Bioinformatics* **27**, 2156-2158.
- 1403 De Gregorio E, Spellman PT, Rubin GM, Lemaitre B (2001) Genome-wide analysis
1404 of the *Drosophila* immune response by using oligonucleotide microarrays.
1405 *Proceedings of the National Academy of Sciences* **98**, 12590.
- 1406 De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaitre B (2002) The Toll and
1407 Imd pathways are the major regulators of the immune response in *Drosophila*.
1408 *The EMBO Journal* **21**, 2568-2579.
- 1409 Dennis AB, Käch H, Vorburger C (in revision) Dual RNA-seq in an aphid parasitoid
1410 reveals plastic and evolved adaptation.
- 1411 Dennis AB, Patel V, Oliver KM, Vorburger C (2017) Parasitoid gene expression
1412 changes after adaptation to symbiont-protected hosts. *Evolution* **71**, 2599-
1413 2617.
- 1414 Dion E, Zélé F, Simon JC, Outreman Y (2011) Rapid evolution of parasitoids when
1415 faced with the symbiont-mediated resistance of their hosts. *Journal of*
1416 *Evolutionary Biology* **24**, 741-750.
- 1417 Dobin A, Davis CA, Schlesinger F, *et al.* (2013) STAR: ultrafast universal RNA-seq
1418 aligner. *Bioinformatics* **29**.
- 1419 Dorémus T, Urbach S, Jouan V, *et al.* (2013) Venom gland extract is not required for
1420 successful parasitism in the polydnavirus-associated endoparasitoid *Hyposoter*
1421 *didymator* (Hym. Ichneumonidae) despite the presence of numerous novel and
1422 conserved venom proteins. *Insect Biochemistry and Molecular Biology* **43**,
1423 292-307.
- 1424 *Drosophila* 12 Genomes C, Clark AG, Eisen MB, *et al.* (2007) Evolution of genes and
1425 genomes on the *Drosophila* phylogeny. *Nature* **450**, 203.
- 1426 Dunn NA, Unni DR, Diesh C, *et al.* (2019) Apollo: Democratizing genome
1427 annotation. *PLoS Comput Biol* **15**, e1006790.
- 1428 Dupas S, Carton Y, Poiriè M (2003) Genetic dimension of the coevolution of
1429 virulence–resistance in *Drosophila* – parasitoid wasp relationships. *Heredity*
1430 **90**, 84.
- 1431 Eddy SR (1998) Profile hidden Markov models. *Bioinformatics* **14**, 755-763.
- 1432 Edgar RC (2004a) MUSCLE: a multiple sequence alignment method with reduced
1433 time and space complexity. *BMC Bioinformatics* **5**, 113.
- 1434 Edgar RC (2004b) MUSCLE: multiple sequence alignment with high accuracy and
1435 high throughput. *Nucleic Acids Research* **32**, 1792-1797.
- 1436 Edgar RC, Myers EW (2005) PILER: identification and classification of genomic
1437 repeats. *Bioinformatics* **21**, i152-i158.
- 1438 Emanuelsson O, Brunak S, von Heijne G, Nielsen H (2007) Locating proteins in the
1439 cell using TargetP, SignalP and related tools. *Nature Protocols* **2**, 953-971.
- 1440 Emms DM, Kelly S (2015) OrthoFinder: solving fundamental biases in whole
1441 genome comparisons dramatically improves orthogroup inference accuracy.
1442 *Genome Biology* **16**, 157.
- 1443 Falabella P, Riviello L, Caccialupi P, *et al.* (2007) A γ -glutamyl transpeptidase of
1444 *Aphidius ervi* venom induces apoptosis in the ovaries of host aphids. *Insect*
1445 *Biochemistry and Molecular Biology* **37**, 453-465.
- 1446 Falabella P, Tremblay E, Pennacchio F (2003) Host regulation by the aphid parasitoid
1447 *Aphidius ervi*: the role of teratocytes. *Entomologia Experimentalis Et*
1448 *Applicata* **97**, 1-9.
- 1449 Finn RD, Bateman A, Clements J, *et al.* (2013) Pfam: the protein families database.
1450 *Nucleic Acids Research* **42**, D222-D230.

- 1451 Flutre T, Duprat E, Feuillet C, Quesneville H (2011) Considering transposable
1452 element diversification in *de novo* annotation approaches. *Plos One* **6**, e16526.
1453 Foerstner KU, von Mering C, Hooper SD, Bork P (2005) Environments shape the
1454 nucleotide composition of genomes. *EMBO reports* **6**, 1208.
1455 Forbes AA, Bagley RK, Beer MA, Hippee AC, Widmayer HA (2018) Quantifying the
1456 unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal
1457 order. *BMC Ecology* **18**, 21.
1458 Gadau J (2009) Phase-unknown linkage mapping in ants. *Cold Spring Harb Protoc*
1459 **2009**, pdb prot5251.
1460 Galtier N, Roux C, Rousselle M, *et al.* (2018) Codon usage bias in animals:
1461 Disentangling the effects of natural selection, effective population size, and
1462 GC-biased gene conversion. *Molecular Biology and Evolution* **35**, 1092-1103.
1463 Geib SM, Liang GH, Murphy TD, Sim SB (2017) Whole genome sequencing of the
1464 Braconid parasitoid wasp *Fopius arisanus*, an important biocontrol agent of
1465 pest teplitid fruit flies. *G3: Genes/Genomes/Genetics* **7**, 2407-2411.
1466 Gerardo NM, Altincicek B, Anselme C, *et al.* (2010) Immunity and other defenses in
1467 pea aphids, *Acyrtosiphon pisum*. *Genome Biology* **11**, R21.
1468 Geuverink E (2017) *Parental and endosymbiont effects on sex determination in*
1469 *haplodiploid wasps : Who is in control?*, University of Groningen.
1470 Geuverink E, Verhulst EC, van Leussen M, van de Zande L, Beukeboom LW (2018)
1471 Maternal provision of non-sex-specific transformer messenger RNA in sex
1472 determination of the wasp *Asobara tabida*. *Insect Molecular Biology* **27**, 99-
1473 109.
1474 Gibson JD, Niehuis O, Verrelli BC, Gadau J (2010) Contrasting patterns of selective
1475 constraints in nuclear-encoded genes of the oxidative phosphorylation
1476 pathway in holometabolous insects and their possible role in hybrid
1477 breakdown in *Nasonia*. *Heredity* **104**, 310.
1478 Godfray HCJ (1994) *Parasitoids: behavioral and evolutionary ecology* Princeton
1479 University Press, Princeton, N.J.
1480 Gold DA, Katsuki T, Li Y, *et al.* (2018) The genome of the jellyfish *Aurelia* and the
1481 evolution of animal complexity. *Nat Ecol Evol*.
1482 Grabherr MG, Haas BJ, Yassour M, *et al.* (2011) Full-length transcriptome assembly
1483 from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**,
1484 644-652.
1485 Greenwood JM, Milutinovic B, Peuss R, *et al.* (2017) Oral immune priming with
1486 *Bacillus thuringiensis* induces a shift in the gene expression of *Tribolium*
1487 *castaneum* larvae. *BMC Genomics* **18**, 329.
1488 Gross P (1993) Insect behavioral and morphological defenses against parasitoids.
1489 *Annual Review of Entomology* **38**, 251-273.
1490 Guindon S, Dufayard J-F, Lefort V, *et al.* (2010) New algorithms and methods to
1491 estimate maximum-likelihood phylogenies: Assessing the performance of
1492 PhyML 3.0. *Systematic Biology* **59**, 307-321.
1493 Guo J, Hatt S, He K, *et al.* (2017) Nine facultative endosymbionts in aphids. A
1494 review. *Journal of Asia-Pacific Entomology* **20**, 794-801.
1495 Haas BJ, Salzberg SL, Zhu W, *et al.* (2008) Automated eukaryotic gene structure
1496 annotation using EVIDENCEModeler and the Program to Assemble Spliced
1497 Alignments. *Genome Biology* **9**, R7.
1498 Hackl T, Hedrich R, Schultz J, Förster F (2014) proofread: large-scale high-accuracy
1499 PacBio correction through iterative short read consensus. *Bioinformatics* **30**,
1500 3004-3011.

- 1501 Hanrahan SJ, Johnston JS (2011) New genome size estimates of 134 species of
1502 arthropods. *Chromosome Res* **19**, 809-823.
- 1503 Harrison MC, Jongepier E, Robertson HM, *et al.* (2018) Hemimetabolous genomes
1504 reveal molecular basis of termite eusociality. *Nature Ecology & Evolution* **2**,
1505 557-566.
- 1506 Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications
1507 through DNA barcodes. *Proceedings. Biological sciences* **270**, 313-321.
- 1508 Heimpel GE, de Boer JG (2007) Sex determination in the Hymenoptera. *Annual*
1509 *Review of Entomology* **53**, 209-230.
- 1510 Heimpel GE, Mills NJ (2017) *Biological control : ecology and applications*.
1511 <http://dx.doi.org/10.1017/9781139029117>
- 1512 Helmkampf M, Cash E, Gadau J (2015) Evolution of the insect desaturase gene
1513 family with an emphasis on social Hymenoptera. *Molecular Biology and*
1514 *Evolution* **32**, 456-471.
- 1515 Henry LM, Roitberg BD, Gillespie DR (2008) Host-range evolution in *Aphidius*
1516 parasitoids: Fidelity, virulence and fitness trade-offs on an ancestral host.
1517 *Evolution* **62**, 689-699.
- 1518 Henter HJ, Via S (1995) The potential for coevolution in a host-parasitoid system. I.
1519 Genetic variation within an aphid population in susceptibility to a parasitic
1520 wasp. *Evolution* **49**, 427-438.
- 1521 Heraty J (2009) Parasitoid biodiversity and insect pest management. *Insect*
1522 *Biodiversity*.
- 1523 Herzog J, Muller CB, Vorburger C (2007) Strong parasitoid-mediated selection in
1524 experimental populations of aphids. *Biol Lett* **3**, 667-669.
- 1525 Hoede C, Arnoux S, Moisset M, *et al.* (2014) PASTEC: An automatic transposable
1526 element classification tool. *Plos One* **9**, e91929.
1527 <https://bipaa.genouest.org> *Bioinformatics Platform for Agroecosystem Arthropods*
1528 *(BIPAA)*. <https://bipaa.genouest.org>
- 1529 Huang H, Wu P, Zhang S, *et al.* (2019) DNA methylomes and transcriptomes analysis
1530 reveal implication of host DNA methylation machinery in BmNPV
1531 proliferation in *Bombyx mori*. *BMC Genomics* **20**, 736.
- 1532 Huang X (1994) On global sequence alignment. *Comput Appl Biosci* **10**, 227-235.
- 1533 Hufbauer RA, Bogdanowicz SM, Harrison RG (2004) The population genetics of a
1534 biological control introduction: mitochondrial DNA and microsatellite
1535 variation in native and introduced populations of *Aphidus ervi*, a parasitoid
1536 wasp. *Molecular Ecology* **13**, 337-348.
- 1537 Jeltsch A, Jurkowska RZ (2014) New concepts in DNA methylation. *Trends in*
1538 *Biochemical Sciences* **39**, 310-318.
- 1539 Jurka J (1998) Repeats in genomic DNA: mining and meaning. *Current Opinion in*
1540 *Structural Biology* **8**, 333-337.
- 1541 Kajitani R, Toshimoto K, Noguchi H, *et al.* (2014) Efficient de novo assembly of
1542 highly heterozygous genomes from whole-genome shotgun short reads.
1543 *Genome Research* **24**, 1384-1395.
- 1544 Kang Z-W, Tian H-G, Liu F-H, *et al.* (2017) Identification and expression analysis of
1545 chemosensory receptor genes in an aphid endoparasitoid *Aphidius gifuensis*.
1546 *Scientific Reports* **7**, 3939.
- 1547 Kassambara A, Mundt F (2016) Factoextra: extract and visualize the results of
1548 multivariate data analyses (ed. package r).

- 1549 Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software
1550 Version 7: Improvements in performance and usability. *Molecular Biology*
1551 *and Evolution* **30**, 772-780.
- 1552 Kavallieratos NG, Tomanovi, x, *et al.* (2004) A survey of aphid parasitoids
1553 (Hymenoptera: Braconidae: Aphidiinae) of Southeastern Europe and their
1554 aphid-plant associations. *Applied Entomology and Zoology* **39**, 527-563.
- 1555 Keilwagen J, Wenk M, Erickson JL, *et al.* (2016) Using intron position conservation
1556 for homology-based gene prediction. *Nucleic Acids Research* **44**, e89-e89.
- 1557 Keller O, Kollmar M, Stanke M, Waack S (2011) A novel hybrid gene prediction
1558 method employing protein multiple sequence alignments. *Bioinformatics* **27**,
1559 757-763.
- 1560 Keller O, Odronitz F, Stanke M, Kollmar M, Waack S (2008) Scipio: using protein
1561 sequences to determine the precise exon/intron structures of genes and their
1562 orthologs in closely related species. *BMC Bioinformatics* **9**, 278-278.
- 1563 Kim BY, Jin BR (2015) Apolipoprotein III from honeybees (*Apis cerana*) exhibits
1564 antibacterial activity. *Comparative Biochemistry and Physiology Part B:*
1565 *Biochemistry and Molecular Biology* **182**, 6-13.
- 1566 Kohany O, Gentles AJ, Hankus L, Jurka J (2006) Annotation, submission and
1567 screening of repetitive elements in Repbase: RepbaseSubmitter and Censor.
1568 *BMC Bioinformatics* **7**, 474.
- 1569 Kolpakov R, Bana G, Kucherov G (2003) mreps: Efficient and flexible detection of
1570 tandem repeats in DNA. *Nucleic Acids Research* **31**, 3672-3678.
- 1571 Koren S, Walenz BP, Berlin K, *et al.* (2017) Canu: scalable and accurate long-read
1572 assembly via adaptive k-mer weighting and repeat separation. *Genome*
1573 *Research* **27**, 722-736.
- 1574 Korf I (2004) Gene finding in novel genomes. *BMC Bioinformatics* **5**, 59.
- 1575 Kraaijeveld AR, Van Alphen JJM, Godfray HCJ (1998) The coevolution of host
1576 resistance and parasitoid virulence. *Parasitology* **116**, S29-S45.
- 1577 Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M (2006) High guanine and
1578 cytosine content increases mRNA levels in mammalian cells. *Plos Biology* **4**,
1579 e180.
- 1580 Kudla G, Murray AW, Tollervey D, Plotkin JB (2009) Coding-sequence determinants
1581 of gene expression in *Escherichia coli*. *Science* **324**, 255.
- 1582 Kuroki S, Matoba S, Akiyoshi M, *et al.* (2013) Epigenetic regulation of mouse sex
1583 determination by the histone demethylase *Jmjd1a*. *Science* **341**, 1106.
- 1584 Kurtz S, Phillippy A, Delcher AL, *et al.* (2004) Versatile and open software for
1585 comparing large genomes. *Genome Biology* **5**, R12.
- 1586 Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature*
1587 *Methods* **9**, 357-359.
- 1588 Leclair M, Pons I, Mahéo F, *et al.* (2016) Diversity in symbiont consortia in the pea
1589 aphid complex is associated with large phenotypic variation in the insect host.
1590 *Evolutionary Ecology* **30**, 925-941.
- 1591 Lee E, Helt GA, Reese JT, *et al.* (2013) Web Apollo: a web-based genomic
1592 annotation editing platform. *Genome Biology* **14**, R93.
- 1593 Lefort V, Longueville J-E, Gascuel O (2017) SMS: Smart Model Selection in PhyML.
1594 *Molecular Biology and Evolution* **34**, 2422-2424.
- 1595 Legeai F, Shigenobu S, Gauthier JP, *et al.* (2010) AphidBase: a centralized
1596 bioinformatic resource for annotation of the pea aphid genome. *Insect*
1597 *Molecular Biology* **19 Suppl 2**, 5-12.

- 1598 Leggett RM, Clavijo BJ, Clissold L, Clark MD, Caccamo M (2014) NextClip: an
1599 analysis and read preparation tool for Nextera Long Mate Pair libraries.
1600 *Bioinformatics* **30**, 566-568.
- 1601 Lemaitre B, Hoffman J (2007) The host defence of *Drosophila melanogaster*. *Ann*
1602 *Rev Immunol* **25**.
- 1603 Letunic I, Bork P (2019) Interactive Tree Of Life (iTOL) v4: recent updates and new
1604 developments. *Nucleic Acids Research* **47**, W256-W259.
- 1605 Li H (2011) A statistical framework for SNP calling, mutation discovery, association
1606 mapping and population genetical parameter estimation from sequencing data.
1607 *Bioinformatics (Oxford, England)* **27**, 2987-2993.
- 1608 Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler
1609 transform. *Bioinformatics* **25**.
- 1610 Li H, Handsaker B, Wysoker A, *et al.* (2009) The Sequence Alignment/Map format
1611 and SAMtools. *Bioinformatics* **25**, 2078-2079.
- 1612 Li Y, Park H, Smith TE, Moran NA (2019) Gene family evolution in the pea aphid
1613 based on chromosome-level genome assembly. *Molecular Biology and*
1614 *Evolution* **36**, 2143-2156.
- 1615 Li Y, Zhang R, Liu S, *et al.* (2017) The molecular evolutionary dynamics of oxidative
1616 phosphorylation (OXPHOS) genes in Hymenoptera. *BMC Evolutionary*
1617 *Biology* **17**, 269.
- 1618 Liepert C, Dettner K (1993) Recognition of aphid parasitoids by honeydew-collecting
1619 ants: The role of cuticular lipids in a chemical mimicry system. *Journal of*
1620 *Chemical Ecology* **19**, 2143-2153.
- 1621 Liepert C, Dettner K (1996) Role of cuticular hydrocarbons of aphid parasitoids in
1622 their relationship to aphid-attending ants. *Journal of Chemical Ecology* **22**,
1623 695-707.
- 1624 Liu N-Y, Wang J-Q, Zhang Z-B, Huang J-M, Zhu J-Y (2017) Unraveling the venom
1625 components of an encyrtid endoparasitoid wasp *Diversinervus elegans*.
1626 *Toxicon* **136**, 15-26.
- 1627 Los DA, Murata N (1998) Structure and expression of fatty acid desaturases.
1628 *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **1394**, 3-
1629 15.
- 1630 Łukasik P, Dawid MA, Ferrari J, Godfray HCJ (2013) The diversity and fitness
1631 effects of infection with facultative endosymbionts in the grain aphid, *Sitobion*
1632 *avenae*. *Oecologia* **173**, 985-996.
- 1633 Luo R, Liu B, Xie Y, *et al.* (2012) SOAPdenovo2: an empirically improved memory-
1634 efficient short-read *de novo* assembler. *GigaScience* **1**, 18-18.
- 1635 Lüthi MN, Vorburger C, Dennis AB (submitted) A novel RNA virus in the parasitoid
1636 wasp *Lysiphlebus fabarum*: genomic structure, prevalence and transmission.
- 1637 Martinez AJ, Kim KL, Harmon JP, Oliver KM (2016) Specificity of multi-modal
1638 aphid defenses against two rival parasitoids. *Plos One* **11**, e0154670.
- 1639 Mateo Leach I, Pannebakker BA, Schneider MV, *et al.* (2009) Thelytoky in
1640 Hymenoptera with *Venturia canescens* and *Leptopilina clavipes* as Case
1641 Studies. In: *Lost Sex: The Evolutionary Biology of Parthenogenesis* (eds.
1642 Schön I, Martens K, Dijk P), pp. 347-375. Springer Netherlands, Dordrecht.
- 1643 Matthey-Doret C, van der Kooij CJ, Jeffries DL, *et al.* (2019) Mapping of multiple
1644 complementary sex determination loci in a parasitoid wasp. *Genome Biology*
1645 *and Evolution*.
- 1646 McCutcheon JP, McDonald BR, Moran N (2009) *Origin of an alternative genetic*
1647 *code in the extremely small and GC-rich genome of a bacterial symbiont.*

- 1648 Miao X, Huang Y, Zhu X, Ding D (2004) A comparative study on development and
1649 reproduction of the parasitoid *Lysiphlebus japonicus* (Hymenoptera:
1650 Aphidiidae) in symbiotic and aposymbiotic host aphids. *Applied Entomology
1651 and Zoology* **39**, 243-248.
- 1652 Miller MA, Schwartz T, Pickett BE, *et al.* (2015) A RESTful API for access to
1653 phylogenetic tools via the CIPRES science gateway. *Evolutionary
1654 bioinformatics online* **11**, 43-48.
- 1655 Mistry J, Finn RD, Eddy SR, Bateman A, Punta M (2013) Challenges in homology
1656 search: HMMER3 and convergent evolution of coiled-coil regions. *Nucleic
1657 Acids Research* **41**, e121-e121.
- 1658 Monticelli LS, Nguyen LTH, Amiens-Desneux E, *et al.* (2019) The preference-
1659 performance relationship as a means of classifying parasitoids according to
1660 their specialization degree. *Evolutionary Applications* **0**.
- 1661 Moreau S, Asgari S (2015) Venom proteins from parasitoid wasps and their biological
1662 functions. *Toxins* **7**, 2385.
- 1663 Morin-Poulard I, Vincent A, Crozatier M (2013) The *Drosophila* JAK-STAT
1664 pathway in blood cell formation and immunity. *JAK-STAT* **2**, e25700.
- 1665 Mugal CF, Arndt PF, Holm L, Ellegren H (2015) Evolutionary consequences of DNA
1666 methylation on the GC content in vertebrate genomes. *G3:
1667 Genes/Genomes/Genetics* **5**, 441.
- 1668 Müller CB, Adriaanse ICT, Belshaw R, Godfray HCJ (2004) The structure of an
1669 aphid–parasitoid community. *Journal of Animal Ecology* **68**, 346-370.
- 1670 Myllymäki H, Valanne S, Rämet M (2014) The *Drosophila* imd signaling pathway.
1671 *The Journal of Immunology* **192**, 3455.
- 1672 NCBI NCfBI, Bethesda (MD) *nr database, available from
1673 ftp.ncbi.nlm.nih.gov/blast/db/*.
- 1674 Nielsen H (2017) Predicting Secretory Proteins with SignalP, available at:
1675 <http://www.cbs.dtu.dk/services/SignalP/>. In: *Protein Function Prediction:
1676 Methods and Protocols* (ed. Kihara D), pp. 59-73. Springer New York, New
1677 York, NY.
- 1678 Nishide Y, Kageyama D, Yokoi K, *et al.* (2019) Functional crosstalk across IMD and
1679 Toll pathways: insight into the evolution of incomplete immune cascades.
1680 *Proceedings of the Royal Society B: Biological Sciences* **286**, 20182207.
- 1681 Nouhaud P, Gautier M, Gouin A, *et al.* (2018) Identifying genomic hotspots of
1682 differentiation and candidate genes involved in the adaptive divergence of pea
1683 aphid host races. *Molecular Ecology* **27**, 3287-3300.
- 1684 Obbard DJ, Shi M, Longdon B, Dennis AB (in revision) A new family of segmented
1685 RNA viruses infecting animals.
- 1686 Oliver KM, Degan PH, Burke GR, Moran NA (2010) Facultative symbionts in
1687 aphids and the horizontal transfer of ecologically important traits. *Annual
1688 Review of Entomology* **55**, 247-266.
- 1689 Oliver KM, Higashi CHV (2018) Variations on a protective theme: *Hamiltonella
1690 defensa* infections in aphids variably impact parasitoid success. *Current
1691 Opinion in Insect Science*.
- 1692 Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial
1693 symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the
1694 National Academy of Sciences* **100**, 1803-1807.
- 1695 Parchman TL, Gompert Z, Mudge J, *et al.* (2012) Genome-wide association genetics
1696 of an adaptive trait in lodgepole pine. *Molecular Ecology* **21**, 2991-3005.

- 1697 Peccoud J, Simon J-C, McLaughlin HJ, Moran NA (2009) Post-Pleistocene radiation
1698 of the pea aphid complex revealed by rapidly evolving endosymbionts.
1699 *Proceedings of the National Academy of Sciences* **106**, 16315.
- 1700 Pennacchio F, Fanti P, Falabella P, *et al.* (1999) Development and nutrition of the
1701 braconid wasp, *Aphidius ervi* in aposymbiotic host aphids. *Archives of Insect*
1702 *Biochemistry and Physiology* **40**, 53-63.
- 1703 Pennacchio F, Strand MR (2006) Evolution of developmental strategies in parasitic
1704 hymenoptera. *Annual Review of Entomology* **51**, 233-258.
- 1705 Perkins DN, Pappin DJC, Creasy DM, Cottrell JS (1999) Probability-based protein
1706 identification by searching sequence databases using mass spectrometry data.
1707 *Electrophoresis* **20**, 3551-3567.
- 1708 Peters RS, Krogmann L, Mayer C, *et al.* (2017) Evolutionary history of the
1709 Hymenoptera. *Current Biology* **27**, 1013-1018.
- 1710 Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest
1711 RADseq: An inexpensive method for *de novo* SNP discovery and genotyping
1712 in model and non-model species. *Plos One* **7**, e37135.
- 1713 Plotkin JB, Kudla G (2011) Synonymous but not the same: the causes and
1714 consequences of codon bias. *Nature reviews. Genetics* **12**, 32-42.
- 1715 Poelman EH, Bruinsma M, Zhu F, *et al.* (2012) Hyperparasitoids use herbivore-
1716 induced plant volatiles to locate their parasitoid host. *Plos Biology* **10**,
1717 e1001435.
- 1718 Poirié M, Colinet D, Gatti J-L (2014) Insights into function and evolution of
1719 parasitoid wasp venoms. *Current Opinion in Insect Science* **6**, 52-60.
- 1720 Porcelli D, Barsanti P, Pesole G, Caggese C (2007) The nuclear OXPPOS genes in
1721 insecta: a common evolutionary origin, a common cis-regulatory motif, a
1722 common destiny for gene duplicates. *BMC Evol Biol* **7**, 215.
- 1723 Poulin R (2007) *Evolutionary Ecology of Parasites (Second Edition)* Princeton
1724 University Press.
- 1725 Powell JR, Moriyama EN (1997) Evolution of codon usage bias in *Drosophila*.
1726 *Proceedings of the National Academy of Sciences* **94**, 7784.
- 1727 Price MN, Dehal PS, Arkin AP (2009) FastTree: Computing large minimum
1728 evolution trees with profiles instead of a distance matrix. *Molecular Biology*
1729 *and Evolution* **26**, 1641-1650.
- 1730 Price MN, Dehal PS, Arkin AP (2010) FastTree 2 – Approximately maximum-
1731 likelihood trees for large alignments. *Plos One* **5**, e9490.
- 1732 Provataris P, Meusemann K, Niehuis O, Grath S, Misof B (2018) Signatures of DNA
1733 methylation across insects suggest reduced DNA methylation levels in
1734 holometabola. *Genome Biology and Evolution* **10**, 1185-1197.
- 1735 Purcell J, Brelsford A, Wurm Y, Perrin N, Chapuisat M (2014) Convergent genetic
1736 architecture underlies social organization in ants. *Current Biology* **24**, 2728-
1737 2732.
- 1738 Quesneville H, Bergman CM, Andrieu O, *et al.* (2005) Combined evidence annotation
1739 of transposable elements in genome sequences. *PLoS Comput Biol* **1**, e22.
- 1740 Quicke DLJ (2014) *The Braconid and Ichneumonid parasitoid wasps : biology,*
1741 *systematics, evolution and ecology.*
1742 <http://public.eblib.com/choice/publicfullrecord.aspx?p=1882154>
- 1743 Ran W, Higgs PG (2010) The influence of anticodon-codon interactions and
1744 modified bases on codon usage bias in bacteria. *Molecular Biology and*
1745 *Evolution* **27**, 2129-2140.

- 1746 Rao YS, Chai XW, Wang ZF, Nie QH, Zhang XQ (2013) Impact of GC content on
1747 gene expression pattern in chicken. *Genetics, selection, evolution : GSE* **45**, 9-
1748 9.
- 1749 Rasekh A, Michaud JP, Kharazi-Pakdel A, Allahyari H (2010) Ant mimicry by an
1750 aphid parasitoid, *Lysiphlebus fabarum*. *J Insect Sci* **10**, 126.
- 1751 Robertson HM, Gadau J, Wanner KW (2010) The insect chemoreceptor superfamily
1752 of the parasitoid jewel wasp *Nasonia vitripennis*. *Insect Molecular Biology* **19**,
1753 121-136.
- 1754 Robertson HM, Waterhouse RM, Walden KKO, *et al.* (2018) Genome sequence of
1755 the wheat stem sawfly, *Cephus cinctus*, representing an early-branching
1756 lineage of the Hymenoptera, illuminates evolution of hymenopteran
1757 chemoreceptors. *Genome Biology and Evolution* **10**, 2997-3011.
- 1758 Rocha EPC, Danchin A (2002) Base composition bias might result from competition
1759 for metabolic resources. *Trends in Genetics* **18**, 291-294.
- 1760 Romero IG, Ruvinsky I, Gilad Y (2012) Comparative studies of gene expression and
1761 the evolution of gene regulation. *Nat Rev Genet* **13**, 505-516.
- 1762 Rouchet R, Vorburger C (2014) Experimental evolution of parasitoid infectivity on
1763 symbiont-protected hosts leads to the emergence of genotype specificity.
1764 *Evolution* **68**, 1607-1616.
- 1765 Sandrock C, Schirrmeister B, Vorburger C (2011) Evolution of reproductive mode
1766 variation and host associations in a sexual-asexual complex of aphid
1767 parasitoids. *BMC Evolutionary Biology* **11**, 348.
- 1768 Sandrock C, Vorburger C (2011) Single-locus recessive inheritance of asexual
1769 reproduction in a parasitoid wasp. *Curr Biol* **21**, 433-437.
- 1770 Scarborough CL, Ferrari J, Godfray HCJ (2005) Aphid protected from pathogen by
1771 endosymbiont. *Science* **310**, 1781.
- 1772 Schmitt-Engel C, Schultheis D, Schwirz J, *et al.* (2015) The iBeetle large-scale RNAi
1773 screen reveals gene functions for insect development and physiology. *Nature*
1774 *Communications* **6**, 7822.
- 1775 Schmitz A, Anselme C, Ravallec M, *et al.* (2012) The cellular immune response of
1776 the pea aphid to foreign intrusion and symbiotic challenge. *Plos One* **7**,
1777 e42114.
- 1778 Sepúlveda D, Zepeda-Paulo F, Ramírez C, Lavandero B, Figueroa C (2016) *Loss of*
1779 *host fidelity in highly inbred populations of the parasitoid wasp Aphidius ervi*
1780 *(Hymenoptera: Braconidae)*.
- 1781 Seward EA, Kelly S (2016) Dietary nitrogen alters codon bias and genome
1782 composition in parasitic microorganisms. *Genome Biology* **17**, 226.
- 1783 Sharma A, Heinze SD, Wu Y, *et al.* (2017) Male sex in houseflies is determined by
1784 *Mdmd*, a paralog of the generic splice factor gene *CWC22*. *Science* **356**, 642.
- 1785 Shi M, Chen X-X (2005) Molecular phylogeny of the Aphidiinae (Hymenoptera:
1786 Braconidae) based on DNA sequences of 16S rRNA, 18S rDNA and ATPase
1787 6 genes. *EJE* **102**, 133-138.
- 1788 Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM (2015)
1789 BUSCO: assessing genome assembly and annotation completeness with
1790 single-copy orthologs. *Bioinformatics* **31**, 3210-3212.
- 1791 Singh R, Bassett E, Chakravarti A, Parthun MR (2018) Replication-dependent histone
1792 isoforms: a new source of complexity in chromatin structure and function.
1793 *Nucleic Acids Research* **46**, 8665-8678.
- 1794 Slater GSC, Birney E (2005) Automated generation of heuristics for biological
1795 sequence comparison. *BMC Bioinformatics* **6**, 31.

- 1796 Šmarda P, Bureš P, Horová L, *et al.* (2014) Ecological and evolutionary significance
1797 of genomic GC content diversity in monocots. *Proceedings of the National*
1798 *Academy of Sciences* **111**, E4096.
- 1799 Smit A, Hubley R, Green P (2013-2015) RepeatMasker Open-4.0.
- 1800 Smith CR, Morandin C, Noureddine M, Pant S (2018) Conserved roles of Osiris
1801 genes in insect development, polymorphism and protection. *Journal of*
1802 *Evolutionary Biology* **31**, 516-529.
- 1803 Smoyer LK, Dorer DR, Nickerson KW, Christensen AC (2003) Phenotype of the
1804 Triplo-lethal locus of *Drosophila melanogaster* and its suppression by
1805 hyperoxia. *Genet Res* **82**, 163-170.
- 1806 Soanes DM, Talbot NJ (2010) Comparative genome analysis reveals an absence of
1807 leucine-rich repeat pattern-recognition receptor proteins in the kingdom Fungi.
1808 *Plos One* **5**, e12725.
- 1809 Sperling P, Ternes P, Zank TK, Heinz E (2003) The evolution of desaturases.
1810 *Prostaglandins, Leukotrienes and Essential Fatty Acids* **68**, 73-95.
- 1811 Strand MR (2014) Teratocytes and their functions in parasitoids. *Current Opinion in*
1812 *Insect Science* **6**, 68-73.
- 1813 Sun Y (2016) sscu: Strength of Selected Codon Usage (ed. 2.6.0 Rpv).
- 1814 Takeda K, Akira S (2005) Toll-like receptors in innate immunity. *International*
1815 *Immunology* **17**, 1-14.
- 1816 Tang H, Zhang X, Miao C, *et al.* (2015) ALLMAPS: robust scaffold ordering based
1817 on multiple maps. *Genome Biology* **16**, 3.
- 1818 Tougeron K, Brodeur J, Le Lann C, van Baaren J (2019) How climate change affects
1819 the seasonal ecology of insect parasitoids. *Ecological Entomology* **0**.
- 1820 Tougeron K, Le Lann C, Brodeur J, van Baaren J (2017) Are aphid parasitoids from
1821 mild winter climates losing their winter diapause? *Oecologia* **183**, 619-629.
- 1822 Tvedte ES, Walden KKO, McElroy KE, *et al.* (2019) Genome of the Parasitoid Wasp
1823 *Diachasma alloeum*, an Emerging Model for Ecological Speciation and
1824 Transitions to Asexual Reproduction. *Genome Biology and Evolution* **11**,
1825 2767-2773.
- 1826 Valanne S, Wang J-H, Rämetsä M (2011) The *Drosophila* toll signaling pathway. *The*
1827 *Journal of Immunology* **186**, 649.
- 1828 Van Oss SB, Carvunis A-R (2019) De novo gene birth. *PLoS Genetics* **15**, e1008160.
- 1829 Van Vaerenbergh M, Debyser G, Devreese B, de Graaf DC (2014) Exploring the
1830 hidden honeybee (*Apis mellifera*) venom proteome by integrating a
1831 combinatorial peptide ligand library approach with FTMS. *Journal of*
1832 *Proteomics* **99**, 169-178.
- 1833 Veleba A, Zedek F, Šmerda J, *et al.* (2016) Evolution of genome size and genomic
1834 GC content in carnivorous holokinetics (Droseraceae). *Annals of Botany* **119**,
1835 409-416.
- 1836 Vieira FG, Forêt S, He X, *et al.* (2012) Unique features of odorant-binding proteins of
1837 the parasitoid wasp *Nasonia vitripennis* revealed by genome annotation and
1838 comparative analyses. *Plos One* **7**, e43034.
- 1839 Vilcinskis A (2016) The role of epigenetics in host-parasite coevolution: lessons
1840 from the model host insects *Galleria mellonella* and *Tribolium castaneum*.
1841 *Zoology* **119**, 273-280.
- 1842 Vilcinskis A (2017) The impact of parasites on host insect epigenetics. *Advances in*
1843 *Insect Physiology*.

- 1844 Vilcinskis A, Vogel H (2016) Seasonal phenotype-specific transcriptional
1845 reprogramming during metamorphosis in the European map butterfly
1846 *Araschnia levana*. *Ecol Evol* **6**, 3476-3485.
- 1847 Vinogradov AE (1998) Genome size and GC-percent in vertebrates as determined by
1848 flow cytometry: The triangular relationship. *Cytometry* **31**, 100-109.
- 1849 Völkl W (1992) Aphids or their parasitoids: Who actually benefits from ant-
1850 attendance? *Journal of Animal Ecology* **61**, 273-281.
- 1851 Völkl W (1997) Interactions between ants and aphid parasitoids: Patterns and
1852 consequences for resource utilization. In: *Vertical Food Web Interactions:
1853 Evolutionary Patterns and Driving Forces* (eds. Dettner K, Bauer G, Völkl
1854 W), pp. 225-240. Springer Berlin Heidelberg, Berlin, Heidelberg.
- 1855 Vorburger C, Gehrler L, Rodriguez P (2010) A strain of the bacterial symbiont
1856 *Regiella insecticola* protects aphids against parasitoids. *Biology Letters* **6**, 109-
1857 111.
- 1858 Vorburger C, Perlman SJ (2018) The role of defensive symbionts in host-parasite
1859 coevolution. *Biological Reviews* **93**, 1747-1764.
- 1860 Walker BJ, Abeel T, Shea T, *et al.* (2014) Pilon: An integrated tool for
1861 comprehensive microbial variant detection and genome assembly
1862 improvement. *Plos One* **9**, e112963.
- 1863 Wang J, Wurm Y, Nipitwattanaphon M, *et al.* (2013) A Y-like social chromosome
1864 causes alternative colony organization in fire ants. *Nature* **493**, 664-668.
- 1865 Wang Y, Jorda M, Jones PL, *et al.* (2006) Functional CpG methylation system in a
1866 social insect. *Science* **314**, 645.
- 1867 Werren JH, Richards S, Desjardins CA, *et al.* (2010) Functional and evolutionary
1868 insights from the genomes of three parasitoid *Nasonia* species. *Science* **327**,
1869 343-348.
- 1870 Wheeler TJ, Eddy SR (2013) nhmmer: DNA homology search with profile HMMs.
1871 *Bioinformatics* **29**, 2487-2489.
- 1872 Wickham H (2007) Reshaping data with the reshape package. *Journal of Statistical
1873 Software; Vol 1, Issue 12 (2007)*.
- 1874 Wickham H (2009) *Ggplot2 elegant graphics for data analysis* Springer, New York.
- 1875 Windsor DA (1998) Controversies in parasitology: Most of the species on Earth are
1876 parasites. *International Journal for Parasitology* **28**, 1939-1941.
- 1877 Wu Y, Bhat PR, Close TJ, Lonardi S (2008) Efficient and accurate construction of
1878 genetic linkage maps from the minimum spanning tree of a graph. *PLoS Genet*
1879 **4**, e1000212.
- 1880 Yamamoto D (2008) Brain sex differences and function of the *fruitless* gene in
1881 *Drosophila*. *Journal of Neurogenetics* **22**, 309-332.
- 1882 Yin C, Li M, Hu J, *et al.* (2018) The genomic features of parasitism, polyembryony
1883 and immune evasion in the endoparasitic wasp *Macrocentrus cingulum*. *BMC
1884 Genomics* **19**, 420.
- 1885 Zepeda-Paulo F, Lavandero B, Mahéo F, *et al.* (2015) Does sex-biased dispersal
1886 account for the lack of geographic and host-associated differentiation in
1887 introduced populations of an aphid parasitoid? *Ecology and Evolution* **5**, 2149-
1888 2161.
- 1889 Zepeda-Paulo FA, Ortiz-Martínez SA, Figueroa CC, Lavandero B (2013) Adaptive
1890 evolution of a generalist parasitoid: implications for the effectiveness of
1891 biological control agents. *Evolutionary Applications* **6**, 983-999.

1892 Zhao C, Escalante Lucio N, Chen H, *et al.* (2015) A massive expansion of effector
1893 genes underlies gall-formation in the wheat pest *Mayetiola destructor*. *Current*
1894 *Biology* **25**, 613-620.

1895
1896

1897

1898

1899

1900

1901

1902

1903

1904

1905

1906

1907

1908

1909

1910

1911

1912

1913

1914

1915

1916

1917

1918 **FIGURES IN MAIN TEXT (title)**

1919

1920 Figure 1. **Aphid parasitoid life cycle:** Generalized life cycle of *Aphidius ervi* and
1921 *Lysiphlebus fabarum*, two different parasitoid wasps that target aphid hosts.

1922

1923 Figure 2. **Codon usage in predicted genes:** Proportions of all possible codons, as used in
1924 the predicted genes in *A. ervi* (top) and *L. fabarum* (bottom). Codon usage was
1925 measured as relative synonymous codon usage (RSCU), which scales usage to the
1926 number of possible codons for each amino acid (RSCU). Codons are listed at the bottom
1927 and are grouped by the amino acid that they encode. The green line depicts GC content
1928 (%) of the codon.

1929

1930 Figure 3. **GC and nitrogen content of expressed genes:** We observe significant
1931 differences (p-values from two-sided t-test) in the GC content between adult and larval
1932 *L. fabarum* in: (A) the most highly expressed 10% of the genes and (B) genes that are
1933 differentially expressed between adults and larvae. In contrast, there is no difference
1934 in the nitrogen content of the same set of genes (C, D).

1935

1936 Figure 4. **Overlap in Venom proteins between *A. ervi* and *L. fabarum*:** Overlap in venom
1937 proteins (A) and venom protein putative function (B) between *A. ervi* and *L. fabarum*

1938

1939 Figure 5: **Phylogeny of hymenopteran GGT sequences.** *A. ervi*/*L. fabarum* and *N.*
1940 *vitripennis*/*P. puparum* venom GGT sequences are marked with blue and orange
1941 rectangles respectively. Letters A, B and C indicate the major clades observed for
1942 hymenopteran GGT sequences. Numbers at corresponding nodes are aLRT values.
1943 Only aLRT support values greater than 0.8 are shown. The outgroup is human GGT6
1944 sequence.

1945

1946

1947

1948

1949

1950

1951

1952

1953

1954

1955

1956 List of additional data files

1957

1958 **Additional Data 1:** details of genetic positions used to construct linkage groups for *L.*

1959 *fabarum*.

1960 **Additional Data 2:** Genbank numbers and taxa information for genome (CDS) graphed

1961 in Supplemental Figure 6.

1962 **Additional Data 3:** file detailing (a) the most highly expressed genes in both taxa and

1963 (b) differential expression between adult and larval *L. fabarum*.

1964 **Additional Data 4:** fasta file of orphan genes for *A. ervi*

1965 **Additional Data 5:** fasta file of orphan genes for *L. fabarum*

1966 **Additional Data 6:** Summary of OMA output, including details of *LRR* genes

1967 **Additional Data 7:** Annotation of venom genes in *L. fabarum* and *A. ervi*

1968 **Additional Data 8:** Details of immune gene annotation

1969 **Additional Data 9:** Expression details of Osiris genes in *L. fabarum* and *A. ervi*

1970 **Additional Data 10:** Details of annotated OXPHOS genes, including duplications in the

1971 assembly

1972 **Additional Data 11:** Details of sex determination gene annotations

1973

1974

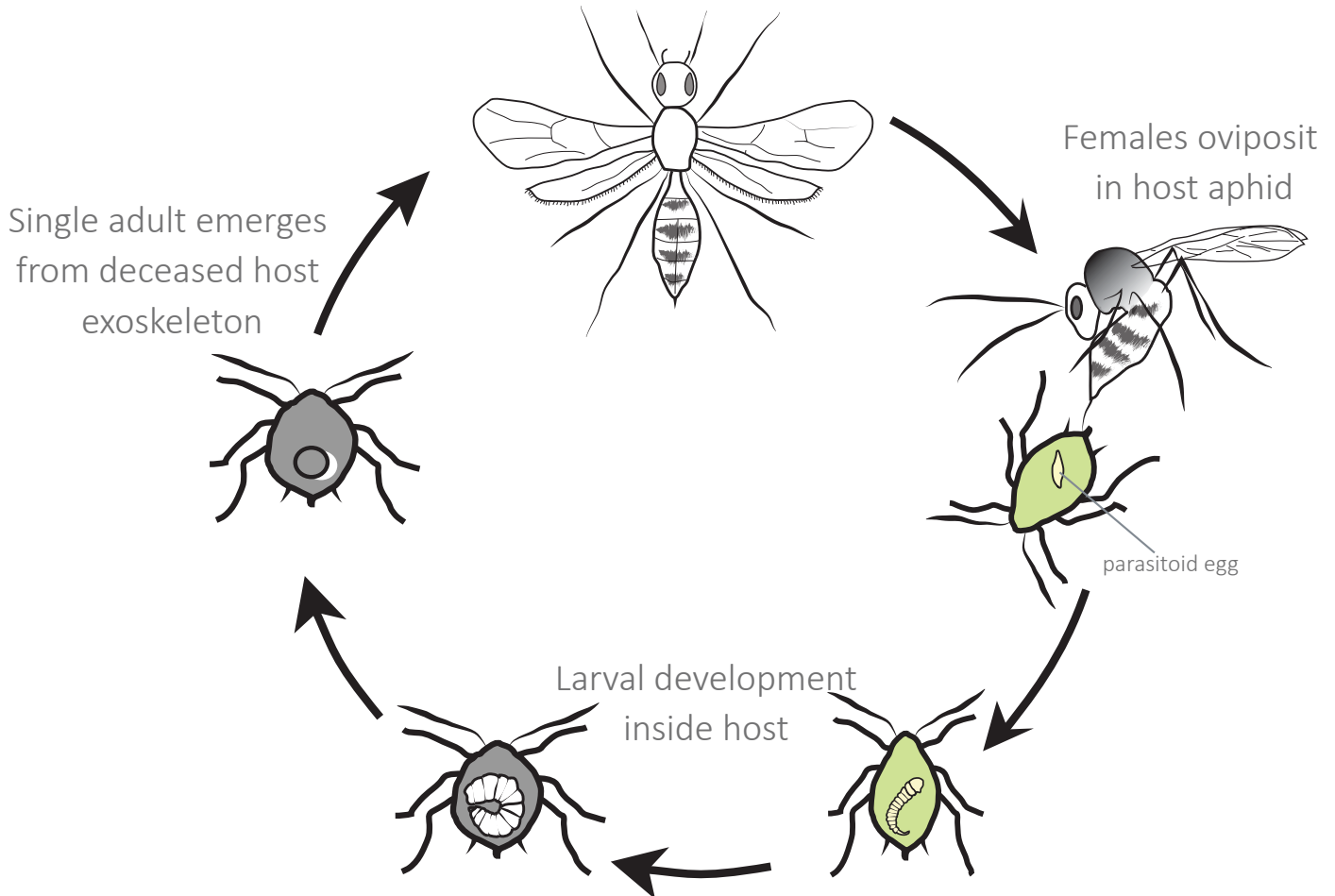
1975

1976

1977

Figure 1

Life cycle of aphid parasitoids



Life history characteristics

	<i>Aphidius ervi</i>	<i>Lysiphlebus fabarum</i>
Host insects	Aphididae	Aphididae
Reproductive mode	Sexual	Asexual or sexual
Host is ant tended	No	Yes, usually
Native range	Europe	Europe
Primary host aphid tribe	Macrosiphini	Aphidini

Figure 2

Codon usage and GC content

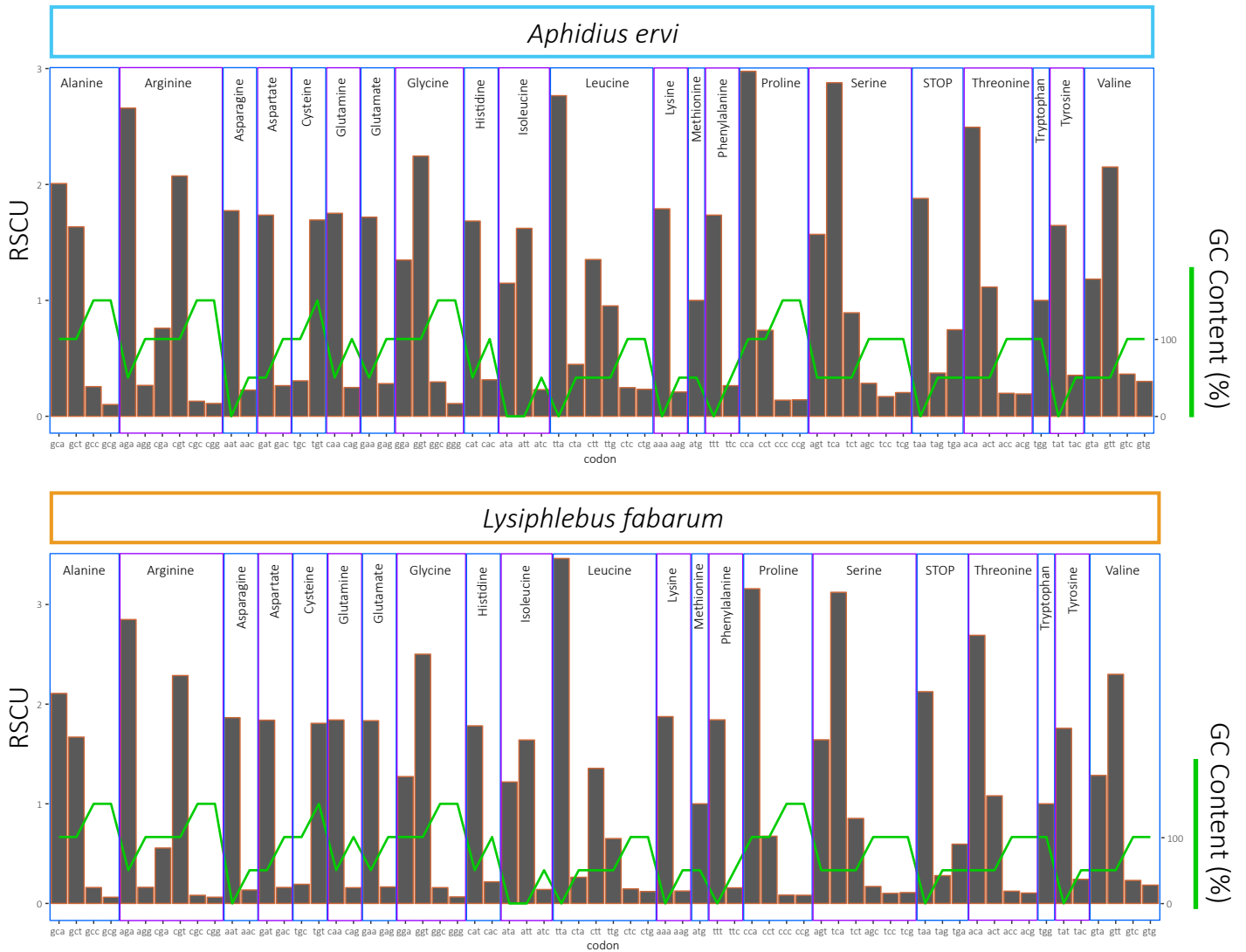


Figure 3

Genes expressed in larval and adult *L. fabarum*

Highly expressed genes

Differentially expressed genes

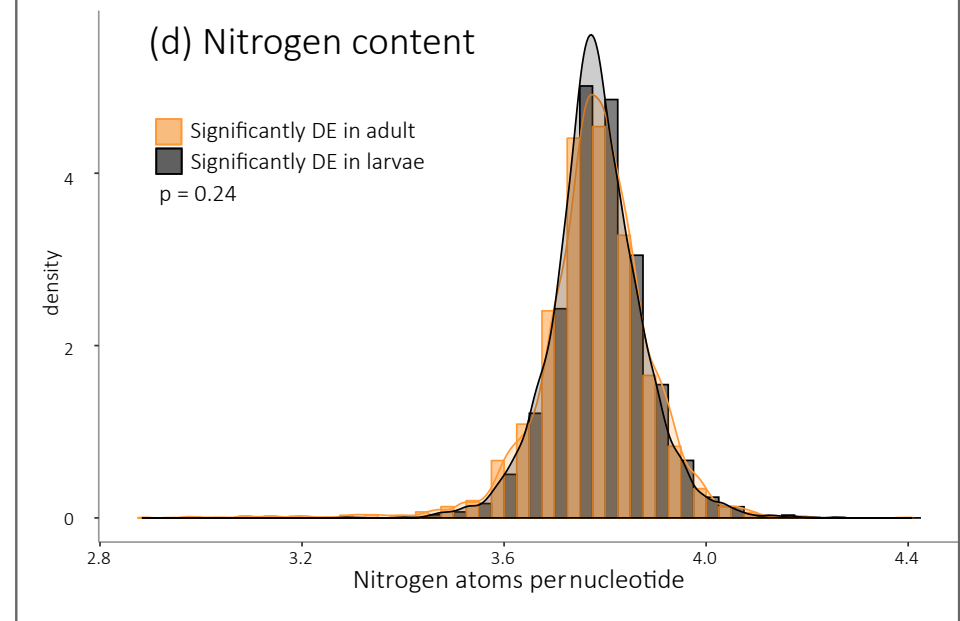
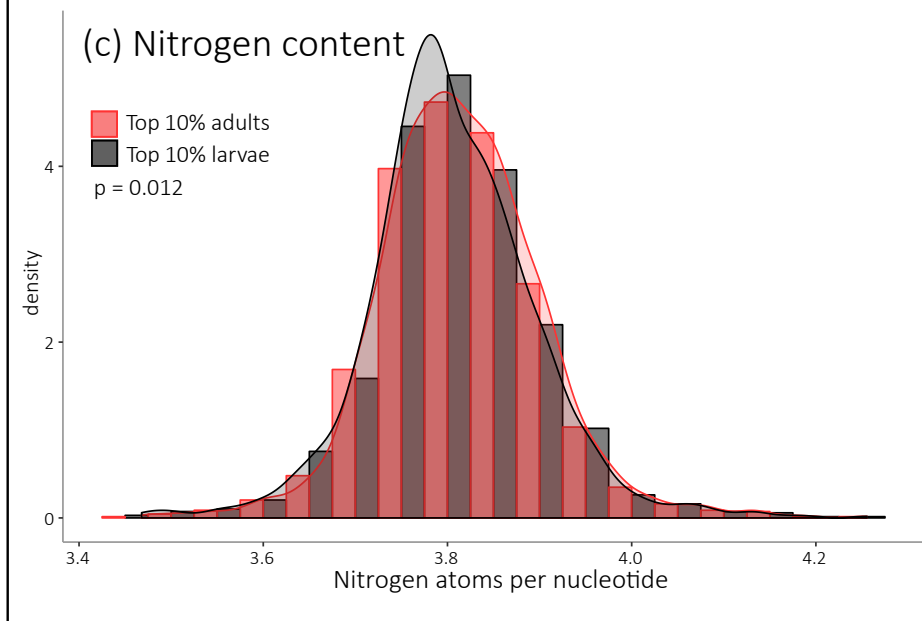
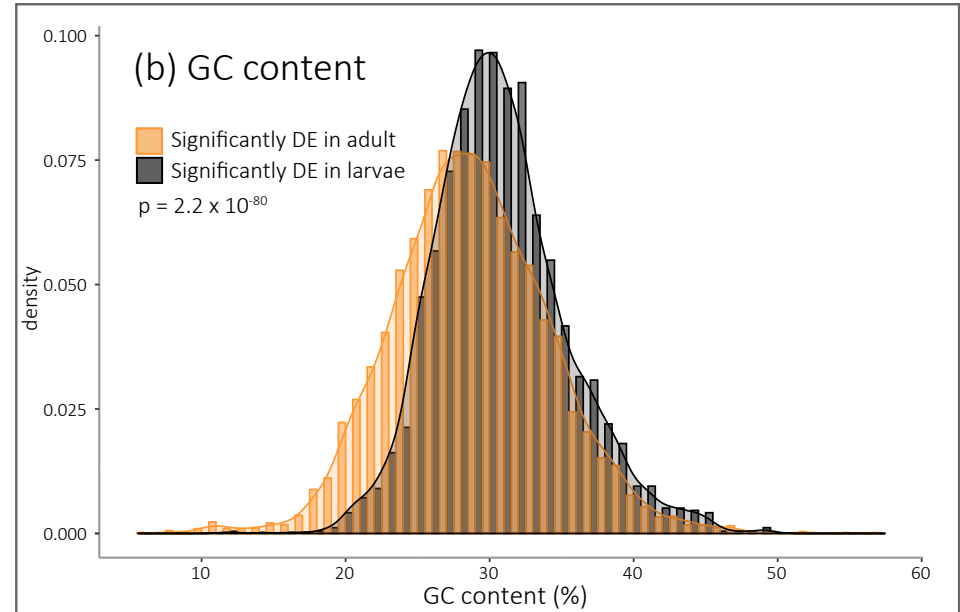
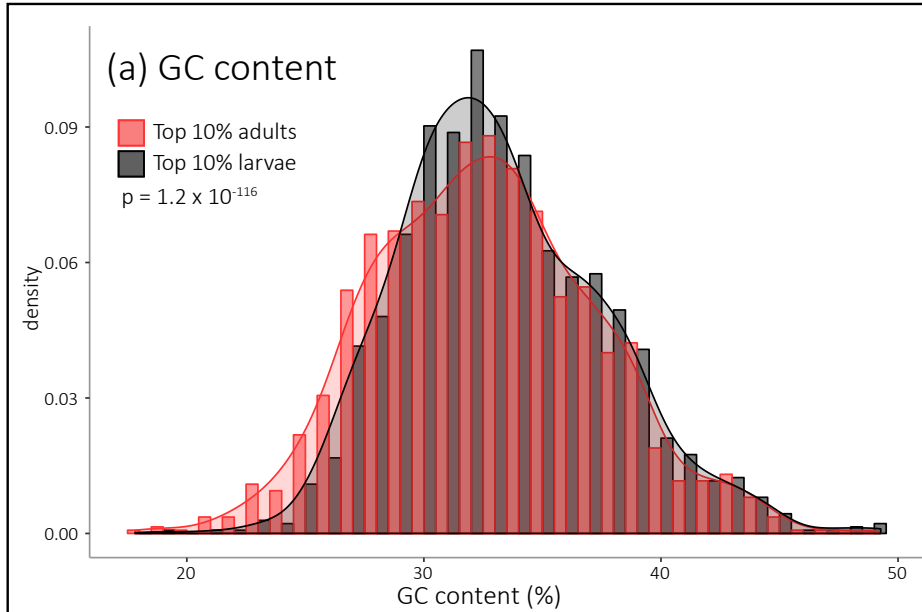


Figure 4

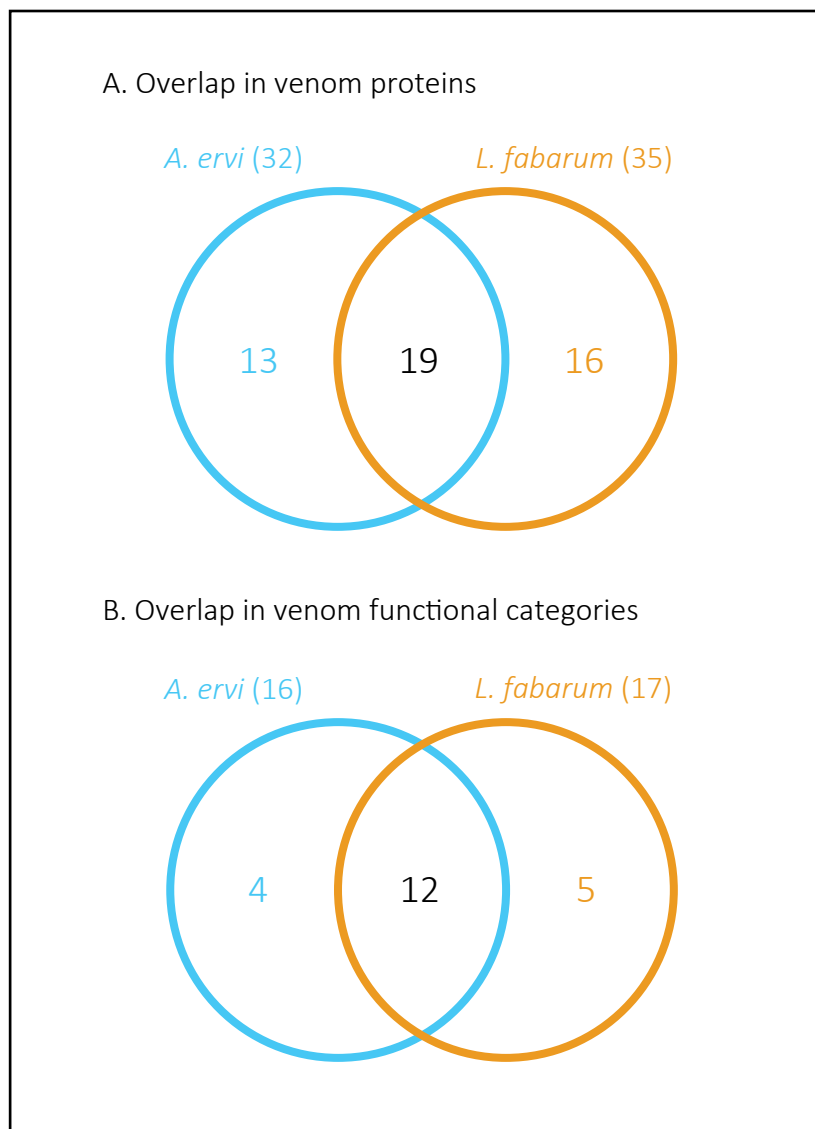


Figure 5

