

Ecological specialization in *Diaeretiella rapae* (Hymenoptera: Braconidae: Aphidiinae) on aphid species from wild and cultivated plants

Y. Navasse^{1,2}, S.A.P. Derocles^{1,2}, M. Plantegenest^{1,2} and A. Le Ralec^{1,2*}

¹INRA-Agrocampus Ouest-Université de Rennes 1, UMR1349 IGEPP, 65 rue de Saint-Brieuc, CS 84215, 35 042 Rennes Cedex, France; ²Université Bretagne Loire, Rennes, France

Abstract

Diaeretiella rapae is an aphid parasitoid with potential for use in biological control strategies. However, several recent genetic studies have challenged the long held view that it is a generalist parasitoid. We investigated its ecological specialization and ability to use resources in cultivated and uncultivated areas. Ecological specialization would reduce its ability to exploit the diversity of aphid species, particularly in uncultivated areas, and to control pest aphids. Four *D. rapae* strains were studied, three reared on pest aphids on Brassicaceae and one strain on a non-pest aphid on Chenopodiaceae. For each strain, we performed host-switching experiments, with a total of six aphid species, five of which *D. rapae* parasitizes in France. We tested cross-breeding ability between strains to detect potential reproductive isolation linked to aphid host species in *D. rapae*. The strain reared on non-pest aphids was able to develop on aphid species from both cultivated and uncultivated plants. The strains reared on pest aphids, however, exclusively parasitized aphid species on cultivated Brassicaceae. In addition, reproductive isolation was detected between strains from uncultivated and cultivated plants. Thus, the *D. rapae* populations examined here appear to be showing ecological specialization or they may even be composed of a complex of cryptic species related to the aphid hosts. The role of *Chenopodium album* as a reservoir for *D. rapae*, by providing a habitat for non-pest aphids on which it can feed, appears to be severely limited, and thus its efficiency to maintain local populations of *D. rapae* in the vicinity of crops is questionable.

Keywords: host range, host switching, reproductive isolation, non-cultivated areas, agroecosystem, *Brevicoryne brassicae*, *Hayhurstia atriplicis*

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Introduction

At the landscape scale, agroecosystems are a mosaic of habitats (Evans *et al.*, 2011; Pocock *et al.*, 2012; Evans *et al.*, 2013) within which there are cultivated and uncultivated

compartments (Woodward & Bohan, 2013; Derocles *et al.*, 2014). The cultivated areas mainly include annual crops, which are harvested seasonally, leading to habitat instability (Rodriguez & Hawkins, 2000; Gurr *et al.*, 2003). In contrast, uncultivated habitats (e.g., field edges, hedges, and woods) are more stable and host a large diversity of plants (Benton *et al.*, 2003). The different characteristics of these two compartments influence the structure and distribution of communities they host in time and space as well as the interactions among organisms (Thompson, 1994; Begon *et al.*, 2006). The exploitation of resources at different trophic levels depends on the degree of

*Author for correspondence:

Phone: +33 (0)2 23 48 56 74

Fax: +33 (0)2 23 48 54 71

E-mail: anne.leralec@agrocampus-ouest.fr

trophic specialization of species (Chaplin-Kramer *et al.*, 2011). Generalist predators or parasitoids species can use a diversity of resources in the mosaic of crops and non-crop habitats. Specialist species, however, are only able to exploit a few patches of resources, dispersed in this mosaic (Tschamntke *et al.*, 2007). Cultivated habitats are abundant but temporary resources for phytophagous insects and their associated natural enemies, while uncultivated habitats can act as refuges for these organisms because they are more stable environments. These habitats provide alternative prey for predators or hosts for parasitoids (Alhmedti *et al.*, 2011; Rusch *et al.*, 2012). Many studies have demonstrated the importance of semi-natural habitats and their alternative resources in agroecosystems in determining the abundance and diversity of pest natural enemies (Tschamntke *et al.*, 2007; Ricci *et al.*, 2009, 2011) and their efficiency for biological control (Langer & Hance, 2004).

The ability to use alternative resources depends on the degree of specialization of the various species involved. Specialist species are more dependent on the availability of a particular prey or host than generalist species. Consequently, specialists may exploit resources in only one type of habitat (cultivated or uncultivated; Gagic *et al.*, 2016) and are prone to local extinction when their main food disappears (Pyke *et al.*, 1977; Holt & Lawton, 1993; Devictor *et al.*, 2010). However, adaptation of specialist species to resources leads to improved performance (Devictor *et al.*, 2010) on these resources, which could lead to a greater efficiency in limiting crop pests. Nevertheless, in some studies, generalist species were demonstrated to be more effective biocontrol agents than specialists (Stiling & Cornelissen, 2005). Species able to exploit at least a small variety of resources can be more efficient than highly specialized ones, as they are able to use resources in non-crop areas. This could ensure population conservation during crop-free periods and increase parasitism pressure when the pest colonizes the crop.

The sub-family Aphidiinae (Hymenoptera: Braconidae) includes about 400 species of aphid parasitoids (Godfray & Shimada, 1999; Sanchis *et al.*, 2001). Most parasitize only one or two aphid species. However, some are able to parasitize more than a dozen aphid species (e.g., *Aphidius ervi* (Haliday, 1834), *Aphidius matricariae* (Haliday, 1834), *Diaeretiella rapae* (M'Intosh, 1855), *Ephedrus plagiator* (Nees, 1811), *Lysiphlebus fabarum* (Marshall, 1896) or *Praon volucre* (Haliday, 1833) (Starý, 2006)). These generalist species may be suitable candidates for regulating pest aphids in conservation biological control strategies as they are theoretically able to exploit both pest and non-pest species.

However, recent reports questioned the actual host range of generalist Aphidiinae. Several studies revealed the existence of cryptic species (i.e., morphologically indistinguishable but genetically different species) among the previously known generalists *A. matricariae* (Derocles *et al.*, 2016), *Binodoxys communis* (Haliday, 1833) (Desneux *et al.*, 2009), *L. fabarum* (Barahoei *et al.*, 2011), and *E. plagiator* (Tomić *et al.*, 2005). Other studies revealed the existence of genetically differentiated populations exploiting different aphid host species, suggesting intraspecific host specialization. For example, Derocles *et al.* (2016) found genetic differentiation within generalist species, such as *D. rapae* and *P. volucre*. In parasitoid species, specialization reduces the number of exploited aphid species and can potentially lead to speciation (Ravigné, 2010).

In this study, we assessed the extent of ecological specialization and reproductive isolation in one of the apparently most generalist Aphidiinae species, *D. rapae* in which genetic

structuring was recently detected. For this, we tested the ability of several parasitoid strains to parasitize aphids from cultivated vs. uncultivated habitats as well as their reproductive compatibility. We chose three aphid species from cultivated plants. Two were from Brassicaceae, the cabbage aphid *Brevicoryne brassicae* (Linnaeus, 1758) and the green peach aphid *Myzus persicae* (Sulzer, 1776), frequently parasitized by *D. rapae* in western France. The other one was the grain aphid *Sitobion avenae* (Fabricius, 1775) from Poaceae, more rarely parasitized in this area. On uncultivated plants, we focused on *Chenopodium album* (Linnaeus, 1753) (Chenopodiaceae). In agricultural areas, this plant is found both in fields and edges, throughout the year, and harbors three potential hosts aphid species *Hayhurstia atriplicis* (Linnaeus, 1761), *Aphis fabae* (Scopoli, 1763), and *M. persicae* (Starý, 2006). Starý & González (1991) suggested that *H. atriplicis* could thus play a role as a reservoir for *D. rapae* in the absence of *B. brassicae*. In addition, Derocles *et al.* (2016) showed that there is genetic differentiation between parasitoids developing on *B. brassicae* or *M. persicae* and those parasitizing *H. atriplicis*. We therefore added *H. atriplicis* and *A. fabae* to the range of tested aphid species.

We tested the hypothesis that uncultivated plants of the genus *Chenopodium* could serve as reservoirs for *D. rapae*. The ability of *D. rapae* to develop on non-pest species in wild plants from semi-natural habitats could improve its efficiency in controlling *B. brassicae* the main pest of cultivated Brassicaceae, by promoting its early presence on Brassica crops (Le Guigo *et al.*, 2012a). The efficiency of *D. rapae* to control *B. brassicae* was recently shown to depend on a quasi-synchronicity between aphid colonization of the plant and parasitoid arrival (Neuville *et al.*, 2016). However, *C. album* could only act as a reservoir for *D. rapae* if there is no ecological specialization or reproductive isolation between individuals developing on *Brassica* and *Chenopodium* aphids. To test these hypotheses, we established four strains of *D. rapae* isolated from three different aphid host species and compared their performances on the selected aphid host species using host switching experiments. We also examined cross-breeding among parasitoid strains from different aphid host species but from the same geographical area, and compared it to cross-breeding among individuals from the same aphid host species but from different geographical areas.

Material and methods

Parasitoids

The aphid parasitoid *D. rapae* is reported to parasitize nearly 100 aphid species around the world (Singh & Singh, 2015). Four strains of *D. rapae* were established from parasitized aphids of its main aphid host species (Němec & Starý, 1984), collected in two geographic areas, western France and Portugal. Three strains were isolated from broccoli fields. Two (one from each locality) were from the cabbage aphid, *B. brassicae*, and one was from the green peach aphid, *M. persicae*, in western France. The last strain was isolated from the aphid *H. atriplicis* collected on *C. album*, in a semi-natural habitat (an herbaceous bank next to a cultivated field) in western France. The sampling locations are shown in table 1. For the three French strains, the maximum distance between the collection sites was approximately 100 km. Each *D. rapae* strain was reared in the laboratory on the aphid species and plant of collection.

Table 1. *Diaeretiella rapae* strains included in this study: name, collection aphid host and plant, collection location, collection year, and number of males and females for breeding.

Name of strains	Aphid host	Plant	Sampling location	Sampling year	Number of individuals to start breeding	
					♀	♂
Dr/Mp	<i>Myzus persicae</i>	<i>Brassica oleracea</i> var. <i>italica</i>	Western France	2009	10	5
Dr/Bb(Fr)	<i>Brevicoryne brassicae</i>	<i>B. oleracea</i> var. <i>italica</i>	Western France	2013	4	5
Dr/Bb(Por)	<i>B. brassicae</i>	<i>B. oleracea</i> var. <i>italica</i>	Portugal	2013	2	1
Dr/Ha	<i>Hayhurstia atriplicis</i>	<i>Chenopodium album</i>	Western France	2013	6	3

Dr/Mp: *D. rapae* from *M. persicae*; Dr/Bb (Fr): *D. rapae* from *B. brassicae* (France); Dr/Bb (Por): *D. rapae* from *B. brassicae* (Portugal); Dr/Ha: *D. rapae* from *H. atriplicis*.

Aphids

Aphid strains used for parasitoid rearing and experiments were established from field-collected individuals or were part of a laboratory clone collection (table 2). Aphids were all maintained on their plant species of collection. Six aphid species were used in the experiments; we chose five species that *D. rapae* is known to parasitize in France and a sixth aphid species not established as part of the *D. rapae* host range. These included: *B. brassicae*, which is the main host of *D. rapae* and a Brassicaceae pest (Němec & Starý, 1984; Wilson & Lambdin, 1987; Pike et al., 1999); *M. persicae* and *H. atriplicis* from which the parasitoid *D. rapae* was collected in the field; and three other species, *A. fabae*, *S. avenae*, and *Acyrtosiphon pisum* (Harris, 1776). *Aphis fabae* and *S. avenae* are part of the theoretical host range of *D. rapae*, while *A. pisum* is not (except for a few records in South America, Singh & Singh, 2015). *B. brassicae*, *H. atriplicis*, *S. avenae*, and *A. pisum* are specialist aphids, on Brassicaceae, Chenopodiaceae, Poaceae, and Fabaceae, respectively. *M. persicae* and *A. fabae* are more generalist species (Blackman & Eastop, 2006). For these two last species, individuals were collected from plants where another *D. rapae* host could be found. This was to test the hypothesis that the parasitoid could use different aphid host species on the same plant species. Thus, *M. persicae* like *B. brassicae* was collected on Brassicaceae, and *A. fabae* like *H. atriplicis* was collected on Chenopodiaceae (table 2).

Three clones of *S. avenae* and *A. pisum* were used because intraspecific variability in host suitability for aphidiinae parasitoids is known for both species (Le Ralec, unpublished data; Simon et al., 1999; Peccoud et al., 2009). As this variability is partially due to symbiotic bacteria (*Hamiltonella defensa*) in *A. pisum* (Dion et al., 2011), we chose three aposymbiotic clones. This was not necessary with *B. brassicae* populations in western France because they are composed of very few genotypes (Le Guigo et al., 2012b), and no protective symbiotic bacteria against parasitoids have ever been found in this species (Henry et al., 2015).

Insect rearing

Aphids and parasitoids were reared under laboratory conditions, at a temperature of 19 ± 2.0 °C, $60 \pm 10\%$ relative humidity and a L16: D8 photoperiod in separate climatic chambers. *B. brassicae* and *M. persicae* were reared separately on broccoli plants (*Brassica oleracea* var. *italica* cv Marathon). The three *S. avenae* clones were reared separately on wheat (*Triticum aestivum* cv. Boston) and the three *A. pisum* clones on alfalfa plants (*Medicago sativa*). Both *H. atriplicis* and

A. fabae were reared on goosefoot plants (*C. album*) obtained from seeds collected in the field.

Host suitability assessments

The ability of each *D. rapae* strain to parasitize the six aphid species was evaluated. The non-host aphid species *A. pisum* was tested to ensure parasitism events did not result from experimental non-choice conditions, regardless of the aphid species. Newly emerged females of each of the four parasitoid strains were fed with honey and water for 24–48 h, and then placed in isolation with a single male of the same strain for 24 h. Mated females had no contact with an aphid prior to the test. Each parasitoid female was tested only once. For each aphid species and clone, ten third instar nymphs were placed on a young plant of the species they had been reared on. After infestation, plants were covered with a Cellophane® bag (29 cm × 15 cm) for 1 h to allow the aphids to settle. They were then exposed to a single *D. rapae* female for 24 h. The female was then removed and the aphids were kept in climatic-controlled chambers for 20 days. For each aphid species, clone and parasitoid strain, ten replicates were carried out. After 10 days, mummified aphids were counted. Experiments were carried out in two distinct sessions (with 10 days between each session). The number, sex ratio (number of females/total number of individuals), and development time of the parasitoids that emerged from the mummies were recorded.

Reproductive compatibility

To assess reproductive compatibility among *D. rapae* strains, originating from various hosts, we performed crosses among the four parasitoid strains. Because Aphidiinae are haplodiploid, the absence of females in the progeny indicates reproductive incompatibility, as 100% male progeny reflects the absence of oocyte fertilization. We used this feature to detect possible reproductive isolation between the strains. To avoid previous mating, mummies were isolated until emergence. Males and females were isolated for 24 h after emergence and supplied with water and honey, and then couples were randomly paired. Ten females from each strain were allowed to mate with ten males of the four possible strains. Five females were proposed to oviposit on aphids belonging to the species of female rearing and five of them on aphids belonging to the species of male rearing. Each mated couple was used only once. We used the same experimental design as above (host suitability experiment) to obtain progeny and check the sex ratio as the proportion of females. The number of

Table 2. Aphid species included in this study: species name based on morphological identification, collection plant, collection location, and collection year.

Species (clone)	Plant	Sampling location	Sampling year
<i>Myzus persicae</i>	<i>Brassica oleracea</i> var. <i>italica</i>	Western France	2006
<i>Brevicoryne brassicae</i>	<i>B. oleracea</i> var. <i>italica</i>	Western France	2006
<i>Aphis fabae</i>	<i>Chenopodium album</i>	Western France	2013
<i>Hayhurstia atriplicis</i>	<i>C. album</i>	Western France	2009
<i>Sitobion avenae</i> (SaR1)	<i>Triticum aestivum</i>	Western France	1990
<i>S. avenae</i> (SaFima5)	<i>T. aestivum</i>	Western France	1990
<i>S. avenae</i> (SaLec14)	<i>T. aestivum</i>	North France	1993
<i>Acythosiphon pisum</i> (pLL01-VF)	<i>Medicago sativa</i>	France	1987
<i>A. pisum</i> (P123)	<i>Pisum sativum</i>	France	1999
<i>A. pisum</i> (JML06)	<i>Medicago lupulina</i>	Germany	2006

mummies, as well as the number and sex of emerged adults were recorded.

Statistical analysis

First, the influence of the aphid species (aphid of collection (rearing) or not), the *D. rapae* strain and the session on the parasitism rate was assessed using a generalized linear model (GLM) assuming a binomial error and a logit link function. Pairwise comparisons between each strain–aphid pair were performed with the function ‘esticon’ using the ‘doBy’ package (Højsgaard, 2004). The effect on the parasitism rate of the aphid species in which the female and male in each cross-breeding have developed was tested with the same method. Second, we determined the effects of aphid host species (aphid of collection (rearing) or not), *D. rapae* strains and session on the development time using an analysis of variance (ANOVA) followed by Tukey’s tests. Finally, the effects of aphid host species (aphid of collection (rearing) or not) and *D. rapae* strains on the sex ratio in both experiments were examined using ANOVA followed by Tukey’s tests. To ensure the accuracy of our model, we verified the normality of the distribution of residuals. All analyses were carried out with R version 3.1.1 (R Development Core team, 2013).

Results

Assessment of host suitability

The four strains differed in the range of aphid species they parasitized and in the parasitism rates obtained on these species (fig. 1). No mummies were obtained on *S. avenae* and *A. pisum*; these two species were removed from the statistical analysis. Parasitoid strains (GLM: $\chi^2 = 14.162$, $df = 3$, $P = 0.0026$), aphid species (GLM: $\chi^2 = 284.571$, $df = 3$, $P < 0.001$), and the interactions between parasitoid strains and aphid species (GLM: $\chi^2 = 248.991$, $df = 9$, $P < 0.001$) significantly influenced the parasitism rate. Higher parasitism rates were obtained on the rearing aphid host species of each strain compared with the other aphid species (GLM: $\chi^2 = 247.6$, $df = 1$, $P < 0.001$). Session had no significant effect (GLM: $\chi^2 = 0.307$, $df = 1$, $P = 0.58$) on the parasitism rate. Overall, mummies were produced on only four aphid species, and the number of aphid species with at least one mummy varied from two to four depending on the parasitoid strain (fig. 1). All the strains parasitized *B. brassicae* and *M. persicae*. Two strains parasitized *H. atriplicis* and one strain parasitized *A. fabae*. The *D. rapae* strain reared on *H. atriplicis* produced offspring on four

aphid species. The three other strains only successfully parasitized *B. brassicae* and *M. persicae*, although a single mummy was obtained from the Portuguese strain on *H. atriplicis*. The strain reared on *H. atriplicis* produced significantly less progeny than the three other strains reared on *B. brassicae* and *M. persicae* (fig. 2).

The development time between egg laying to adult emergence differed significantly depending on the parasitoid strains (ANOVA: $F(3, 287) = 27.85$, $P < 0.001$), aphid species (ANOVA: $F(3, 287) = 10.51$, $P < 0.001$), and the interaction between these factors (ANOVA: $F(4, 287) = 3.39$, $P = 0.009$) (fig. 1). The strain reared on *H. atriplicis* required 1 or 2 more days to complete its development compared with the strains reared on *M. persicae* and *B. brassicae* (fig. 1). Finally, for all strains, the progeny sex ratio ranged from 0.7 to 1 (fig. 1), and neither the aphid species (ANOVA: $F(3, 63) = 0.95$, $P = 0.42$) or the strains (ANOVA: $F(3, 63) = 2.14$, $P = 0.1$) had a significant effect.

Reproductive compatibility

The overall sex ratio of the progeny was approximately 0.8 females, except for crosses involving individuals reared on *H. atriplicis* mated with a partner from another strain. In that case, only males were obtained (fig. 3). The parasitism rates of the four *D. rapae* strains on the different aphid species observed in this experiment were similar to those obtained in the previous experiment (host suitability assessment) (fig. 3). In all cross-breeds, the parasitism rate was significantly higher on the female’s rearing aphid host species compared with the other aphid species (GLM: $\chi^2 = 209.71$, $df = 1$, $P < 0.001$; fig. 3). Mummies were only produced on *H. atriplicis* when the female belonged to the *H. atriplicis* strain.

Discussion

The aphid parasitoid *D. rapae* is known to parasitize 98 aphid species (Singh & Singh, 2015). However, previous studies found local variations in its host range (Le Ralec *et al.*, 2011). In the present study, we investigated the level of host (aphid species) specialization of four strains reared from three aphid species and two geographical origins. An increased level of specialization was observed in the three *D. rapae* strains collected on the aphid species, which infest crops (*B. brassicae* from two different areas and *M. persicae*); whereas the *D. rapae* strain isolated from the non-pest aphid *H. atriplicis* was able to parasitize a wider range of aphid species. In addition, this strain appeared reproductively isolated from the

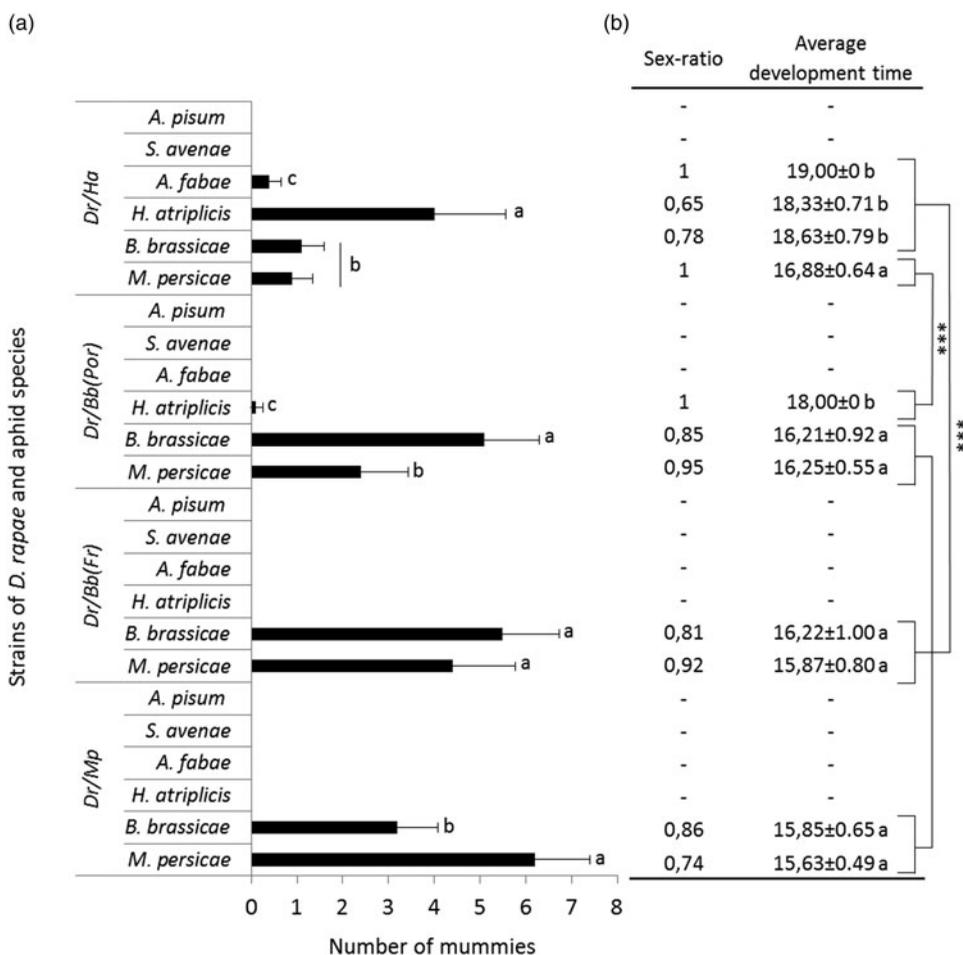


Fig. 1. Total number of mummies produced per aphid species by the various *Diaeretiella rapae* strains. (a) Average number of mummies per female with standard deviation; the sex ratio is expressed as the female ratio and the mean development time in days with standard deviation for each pair of parasitoid strains and aphid species (b). Dr/Mp: *D. rapae* from *Myzus persicae*; Dr/Bb(Fr): *D. rapae* from *Brevicoryne brassicae* (France); Dr/Bb(Por): *D. rapae* from *B. brassicae* (Portugal); Dr/Ha: *D. rapae* from *Hayhurstia atriplicis*. The data were analyzed with a generalized linear model for parasitism rate and ANOVA for development time with pairwise comparisons with 'esticon' and Tukey's tests as *post hoc* tests, respectively. Results from the three clones of *Acythosiphon pisum* and *Sitobion avenae* were pooled as no mummies were obtained. Significant differences are indicated with different letters ($P < 0.05$) and with asterisks ($***P < 0.001$).

three others. These results suggest that specialized cryptic species exist among populations of this presumed generalist parasitoid. The role of *H. atriplicis* in maintaining *D. rapae* populations when pest aphids are absent is therefore questionable.

The four strains of *D. rapae* studied here had different host ranges among the tested aphid species, but also produced varying numbers of offspring on each suitable aphid species. First, the three strains from *B. brassicae* and *M. persicae* only parasitized these two aphid host species, regardless of their geographical origin. Next, the strain from *H. atriplicis* parasitized four of the six aphid species tested. In addition to its host of origin, the strain from *H. atriplicis* successfully developed on *B. brassicae*, *M. persicae*, and *A. fabae*, the latter species was collected on *C. album* as well as *H. atriplicis*. *B. brassicae* and *M. persicae* are very common aphids in European agroecosystems (Blackman & Eastop, 2000), and constitute an abundant resource during a short period of time for *D. rapae* on cultivated Brassicaceae (e.g., oil seed rape, cabbage, turnip). In

contrast, *H. atriplicis* is a scarce resource for a parasitoid because it is only present for three months (August to October), on wild Chenopodiaceae (Blackman & Eastop, 2000), and in small abundance in agroecosystem. We can hypothesize that it is highly risky for a parasitoid species to specialize on a scarce resource. However, the ability to parasitize other aphid species on the same plant, such as *A. fabae* or *M. persicae*, and also aphid species exploiting plants of other families (e.g., *B. brassicae* and *M. persicae* on Brassicaceae), could limit the risks associated with specialization. The parasitic success of the most generalist strain (from *H. atriplicis*) was lower than the parasitic success of the more specialized strains, on the shared aphid host species *B. brassicae* and *M. persicae*. This reduction in parasitism efficacy is expected as a result of a trade-off between the ability to exploit numerous aphid species and parasitism efficacy (Devictor *et al.*, 2010; Le Ralec *et al.*, 2010; Gagic *et al.*, 2016).

The grain aphid *S. avenae* was never parasitized, even though this species was previously reported to belong to the

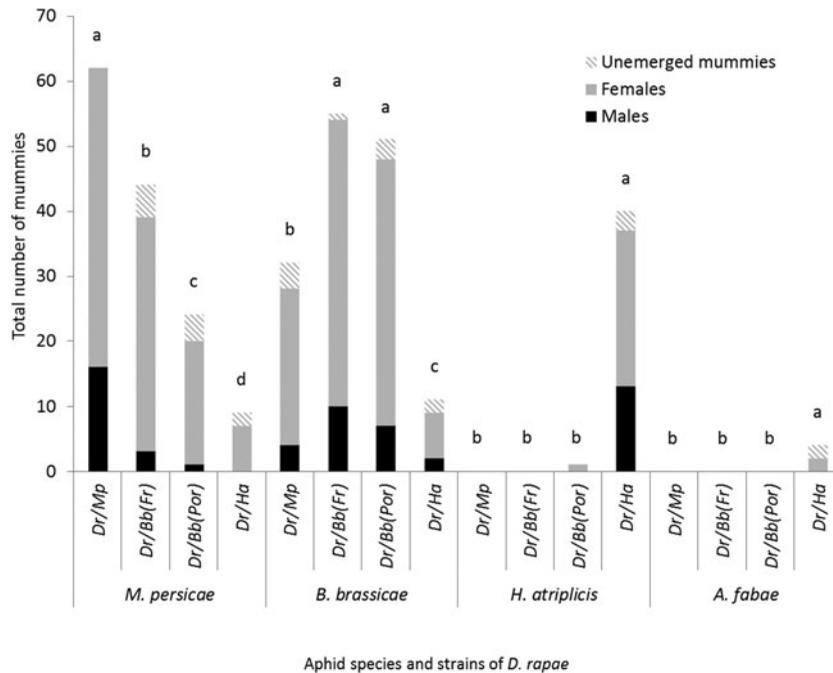


Fig. 2. Total number of adult female and male offspring, as well as unemerged mummies, produced per aphid species by the various *Diaeretiella rapae* strains. The data were analyzed with a generalized linear model with pairwise comparisons with an 'esticon' *post hoc* test. Different letters show significant differences ($P < 0.05$).

D. rapae host range (Pike *et al.*, 1999; Singh & Singh, 2015). Such an unsuitability of theoretical *D. rapae* hosts was previously observed for other cereal aphids (Antolin *et al.*, 2006; Le Ralec *et al.*, 2011). Unfortunately, we did not find any *D. rapae* individuals from cereal aphids to use as an experimental strain. Less surprisingly, no progeny were obtained on the pea aphid *A. pisum*, used as a non-host control in this study, even in the experimental non-choice conditions.

The highest parasitic success was always obtained on the aphid host species of collection, which was also the rearing host. This is not surprising as strains collected on a given host are expected to be adapted to this aphid species. This variation in performance also indicates some degree of host specialization of the populations in the field (Henry *et al.*, 2008). Differences in performance might also result from parasitoid strains being continuously maintained on their aphid species of collection since sampling. Rearing on a single aphid host species was shown to have a positive impact on parasitoid success (Vafaie *et al.*, 2013), enhance host recognition (Wickremasinghe & Van Emden, 1992; Storeck *et al.*, 2000), and induce preference in host choice (Van Emden *et al.*, 2008).

The observed differences in the set of aphid host species successfully parasitized by the four *D. rapae* strains are consistent with the observations of Derocles *et al.* (2016). Indeed, using molecular phylogenetic analysis, Derocles *et al.* (2016) showed a clear genetic differentiation between *D. rapae* populations collected on *B. brassicae* and *M. persicae* and populations sampled on *H. atriplicis* from the same geographical area. Our results suggest that this molecular variation between *D. rapae* populations from different aphid species actually reflects host specialization. In addition, the strains from *B. brassicae* and *M. persicae* appeared to be reproductively incompatible with the strain from *H. atriplicis*. While distant populations from France

isolated from *B. brassicae* or *M. persicae* and Portugal from *B. brassicae* were able to successfully interbreed, the French population reared on *H. atriplicis* did not produce female offspring when they mated with another strain. Our data reveal a clear convergence between the observed genetic differentiation, host specialization, and reproductive isolation in the populations of the generalist parasitoid *D. rapae*. Such cryptic species or specialized biotypes are known in other Aphidiinae. In several studies, morphometric analysis revealed specialized biotypes (Tomić *et al.*, 2005; Žikić *et al.*, 2009; Barahoei *et al.*, 2011; Tomanović *et al.*, 2014). However, in our case, a morphological re-examination of *D. rapae* individuals based on the main morphological characteristics usually used to identify Aphidiinae (maxillary palps, number of antennal segments, size, shape, and rib wings and the anterolateral area of the petiole; Kavallieratos *et al.*, 2001; Tomanović *et al.*, 2003; Kavallieratos *et al.*, 2005; Tomanović *et al.*, 2007) failed to find any evidence of differentiation between strains.

Derocles *et al.* (2016) could not conclude in favor of two different species among *D. rapae* on the sole basis of molecular variations. Indeed, the analytical approach they applied, which was based on a combination of maximum likelihood trees and the species delimitation method called Bayesian Poisson tree processes model (Zhang *et al.*, 2013), did not separate the two genetic sub-groups (*D. rapae* populations from *H. atriplicis* on one side and populations from other species on the other) into distinct species. However, our complementary findings showing that morphologically indistinguishable (based on our morphological re-examination) host-specialized strains are reproductively isolated strongly suggest that there are at least two cryptic *D. rapae* species. Desneux *et al.* (2009) reported similar results and described a new species, *Binodoxys koreanus*,

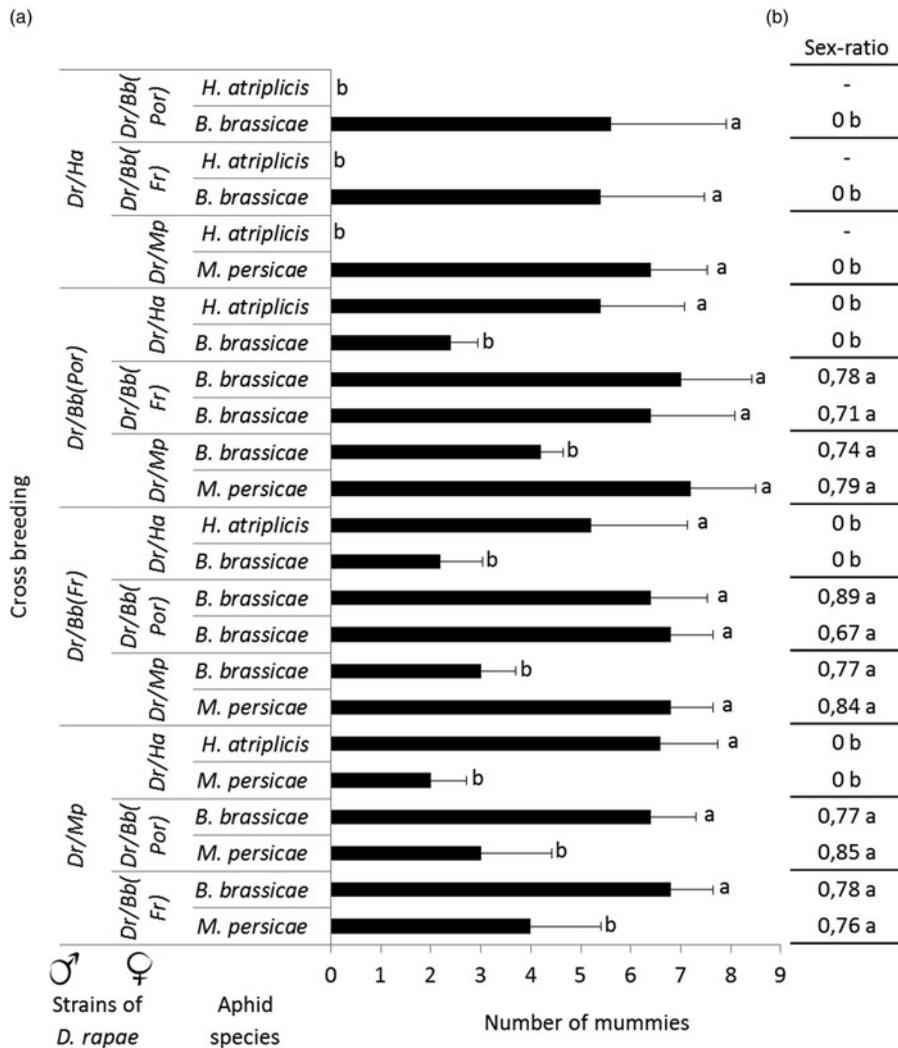


Fig. 3. Average numbers of mummies produced (with standard deviation) (a) and offspring sex ratio (as the female ratio) (b) for each aphid species tested and for all types of cross-breeding. Dr/Mp: *Diaeretiella rapae* from *Myzus persicae*; Dr/Bb: *D. rapae* from *Brevicoryne brassicae*; Dr/Bb (Por): *D. rapae* from *B. brassicae* (Portugal); Dr/Ha: *D. rapae* from *Hayhurstia atriplicis*. The data were analyzed with a generalized linear model for parasitism rate with pairwise comparisons with an 'esticon' *post hoc* test. To analyse the sex ratio, we used ANOVA with Tukey's tests; different letters indicate significant differences ($P < 0.05$).

from two populations previously identified as *B. communis*. Consequently, our results suggest that a new *Diaeretiella* species could potentially be described. The evolutionary process leading to reproductive isolation of individuals from the same ancestral species and resulting in two species (Ravigné, 2010) involves physical or biological reproductive barriers. In the case of *B. communis* and *B. koreanus* (Desneux *et al.*, 2009), the two species originated from distant countries and their recent divergence could be explained by geographical isolation. In the present study, the incompatible strains were collected in the same geographical area. Sympatric speciation, i.e., the emergence of 'new species' without geographical isolation (Futuyma, 2008; Wiley & Lieberman, 2011) can result from micro-habitat differentiation, which leads to a reduction in gene flow between specialized populations.

Most aphid species are specialized on a few plant species or families (Ferrari *et al.*, 2008); thus, we hypothesize that habitat

choice could be the primary driver of parasitoid specialization (Le Ralec *et al.*, 2010). Indeed, plants emit secondary compounds during aphid attack that attract various Aphidiinae parasitoids (Guerrieri *et al.*, 1993; Funk *et al.*, 2002; Figueroa *et al.*, 2004; Toneatto *et al.*, 2010) and thus affect the aphid host species selection behavior of the parasitoid (Rehman & Powell, 2010). Similarly, after pest attack, Brassicaceae emit secondary compounds that attract *D. rapae* (Blande *et al.*, 2007; Pope *et al.*, 2008). We propose that the secondary compounds Chenopodiaceae emit when infested with aphids are different. These differences in compounds potentially lead to specialization among sections of the population for the specific recognition of these compounds.

According to our results, the presumed generalist species *D. rapae* could thus comprise at least two biotypes or even distinct species, one developing on aphid species infesting Brassicaceae and one on aphid species on Chenopodiaceae.

The synonymy of the species name *D. rapae* with names referring to Chenopodiaceae, such as *Diaeretiella chenopodii* or *Diaeretiella chenopodiaphidis* (Singh & Singh, 2015) should therefore be re-examined. The theoretical *D. rapae* host range includes almost nearly 100 aphid host species and similar cases of specialization in this parasitoid have been shown for other aphid hosts (see Antolin *et al.*, 2006; Le Ralec *et al.*, 2011). The potential number of specialized sub-taxa or cryptic species could then be greater. This study only included four populations with probably low genetic diversity due to the low number of individuals used to constitute the strains. To fully elucidate the taxonomic status of *D. rapae*, we need to conduct host switching and cross-breeding experiments on a larger number of populations originating from a wider range of aphid species, plants, and countries.

Finally, according to our findings, the role of *H. atriplicis* as a reservoir for *D. rapae* as proposed by Némec & Starý (1984) and Starý & González (1991) is questionable. We found an asymmetry in host range between specialist strains from *B. brassicae* and *M. persicae* and a more generalist strain from *H. atriplicis*, with the former strains being unable to develop on *H. atriplicis*, a situation not tested by Starý & González (1991). This confirms the scarcity of parasitoid exchanges between cultivated and uncultivated compartments (Derocles *et al.*, 2014). Promoting non-pest aphids on non-crop plants as a parasitoid reservoir may not be a suitable way of enhancing natural pest control by *D. rapae* in conservation biological control. Wild Brassicaceae species in the vicinity of the field are more likely to be the actual reservoir of the corresponding *D. rapae* sub-taxa in the studied area, as they harbor the pest aphid species *B. brassicae* or *M. persicae* (Le Guigo *et al.*, 2012a, b). However, wild Brassicaceae in uncultivated compartments in agroecosystems could also act as a pest reservoir.

Our study is consistent with several recent studies, which demonstrated that populations of Aphidiinae species generally considered as generalists may potentially include specialized sub-groups or even cryptic species, as seems to be the case for *D. rapae*. This has to be fully considered in ecological studies on interaction networks between plants, aphids, and parasitoids to avoid misinterpretation of resource sharing (Gagic *et al.*, 2016) and the structure of ecological networks (Derocles *et al.*, 2015). Furthermore, the importance of aphid traits in the evolution of host specificity in Aphidiinae is often highlighted (Raymond *et al.*, 2016). Nevertheless, the plant taxa supporting the aphid hosts could also be a key factor driving parasitoid specialization.

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