

Asexuality cured: the relations and differences between sexual and asexual *Apoanagyrus diversicornis*

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We investigated whether asexual (thelytokous) *Apoanagyrus diversicornis* and sexual (arrhenotokous) *A. diversicornis* (Hymenoptera: Encyrtidae) belong to the same biological species. The strains are allopatric and there are no morphological differences. The sexual *A. diversicornis* strain originates from south-western Brazil. It was introduced into Africa for the biological control of the cassava mealybug *Phenacoccus manihoti*. The asexual strain parasitizes *P. herreni* in Colombia. Feeding antibiotics to asexual *A. diversicornis* or rearing parasitized hosts at 33 instead of 25°C led to male offspring production. These males could be crossed with virgin females of the sexual strain, leading to fertile female (i.e. hybrid) offspring. There were no indications of hybrid breakdown. Thus, asexuality in *A. diversicornis* is caused by microorganisms, can be cured, and the two strains belong to the same biological species. The microorganisms associated with thelytoky in *A. diversicornis* belong to the genus *Wolbachia*. There were differences between *A. diversicornis* strains and between host species in survival probability from egg to adult. In addition, some mortality was associated with the feeding of tetracycline and with the production of males, but not with rifampicin, sulphadiazine (which was not effective), or a temperature increase from 25 to 33°C. Survival of hybrids was intermediate between the survival probabilities of the parental strains. Male behaviour and function were not different between the strains, but female behaviour clearly was. Females of the thelytokous strain were less often inseminated by males of either strain than were arrhenotokous females. This suggests a difference in the rate of decay between expressed traits (female sexual behaviour, which is possibly selected against) and traits that are not expressed (male sexual behaviour and function).

Keywords: microbial infection, parthenogenesis, sexual behaviour decay, taxonomy, thelytoky, *Wolbachia*.

Introduction

Thelytoky is the production of only female offspring in hymenopterous parasitoids by way of parthenogenesis. Males may occasionally be observed in thelytokous populations, sometimes induced by high temperatures (over 30°C) (e.g. Gordh & Lacey (1976); Laraichi (1978)). The 'normal' mode of reproduction in the Hymenoptera is arrhenotoky. Males, arising from unfertilized eggs, are haploid, whereas females are diploid and arise from fertilized eggs. The terms 'asexual', 'parthenogenetic' and 'uniparental' are used synonymously with 'thelyto-

kous' in the Hymenopteran literature, and 'sexual' and 'biparental' are synonymous with 'arrhenotokous'. Arrhenotokous and thelytokous females can be distinguished by the sex of offspring of virgin females: male in arrhenotokous, female in thelytokous strains.

Recently, thelytoky has been recognized to be frequently associated with infection of the parasitoid by rickettsias of the genus *Wolbachia*. The *Wolbachia* are cytoplasmically inherited through eggs, but not through sperm. Male hosts therefore constitute a dead end: *Wolbachia* cannot reproduce beyond the life of a male host. *Wolbachia* advance their own transmission in a number of ways by causing their host to produce more female offspring than unin-

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fectured individuals. Apart from cytoplasmic incompatibility (Breeuwer *et al.*, 1992; O'Neill *et al.*, 1992) or a change of genotypic males into phenotypic females (Rousset *et al.*, 1992) (which will not be discussed here), *Wolbachia* can cause complete thelytoky in parasitoid wasps (Stouthamer *et al.*, 1993; Werren *et al.*, 1995). Such infected wasps produce daughters from unfertilized eggs. They can be 'cured' (i.e. rendered arrhenotokous) by treatment with antibiotics or high temperatures, which kills the microorganisms (Stouthamer *et al.*, 1990a) and causes the females to produce male offspring. There are also forms of asexuality, however, which are not associated with microorganisms (Stouthamer *et al.*, 1990b).

Some parasitoid species consist of both thelytokous and arrhenotokous individuals. Examples are *Apoanagyrus diversicornis* (Howard) [previously *Epidinocarsis diversicornis* (Howard) (Noyes & Hayat, 1994)], where (as far as we know) arrhenotokous and thelytokous populations are geographically separated (Pijls & van Alphen, 1996), and several *Trichogramma* spp., in which arrhenotokous and thelytokous individuals may co-occur in the same locality (Stouthamer *et al.*, 1990b). The thelytokous *Trichogramma* strains referred to are infected with *Wolbachia*. In other species, infection with microorganisms has gone to fixation and only females exist. Examples of the latter are *Encarsia formosa* (Zchori-Fein *et al.*, 1992), *Leptopilina australis* and *L. clavipes* (I.E.M. Eijs, pers. comm.).

Apoanagyrus diversicornis (Howard) (Hymenoptera: Encyrtidae) is a solitary endoparasitoid of mealybugs. It is native of South America. In Colombia, a thelytokous strain is known from the mealybug *Phenacoccus herreni* Cox and Williams (Homoptera: Pseudococcidae) (Van Driesche *et al.*, 1987). A strain with an arrhenotokous mode of reproduction was found on the closely related mealybug *P. manihoti* Matile-Ferrero in Paraguay and south-western Brazil (Löhr *et al.*, 1990). Over the last decade, attempts have been made to introduce the south-west Brazilian strain into Africa in addition to the congeneric sympatric endoparasitoid *A. lopezi* De Santis, for the biological control of the cassava mealybug *P. manihoti* (Herren & Neuenschwander, 1991). The *A. diversicornis* strains will henceforth be abbreviated TD for the (thelytokous) strain from Colombia and AD for the (arrhenotokous) strain from southwestern Brazil. There are no morphological differences between AD and TD (J.S. Noyes, pers. comm.).

The interest in this system arose partly from applied research, where we wanted to know whether

the strains are indeed the same species (Pijls & van Alphen, 1996). The systematics of thelytokous populations have been problematic because it was not possible to use crosses to determine the relationship between populations. Naming them separately solely because of their mode of reproduction did not seem appropriate (J.S. Noyes, pers. comm.). The occasional occurrence of males in our culture of the thelytokous strain during spells of hot weather indicated that the asexuality might be curable, because it might be caused by a microbial infection. If we could obtain males by curing TD females, we would be able to test if TD and AD are the same biological species.

We investigated how thelytoky in *A. diversicornis* is caused, and whether TD and AD can interbreed and produce viable F₁ and F₂ progeny. Differences in behaviour or physiology between arrhenotokous and thelytokous strains may reflect long-term effects of a possible *Wolbachia* infection or of reproductive isolation. We compared the mating ability and the offspring survival of TD and AD in *P. herreni* and *P. manihoti*, the known hosts of the strains.

Materials and methods

Cultures

Cassava plants (*Manihot esculenta* Crantz) were reared in a greenhouse from cuttings from Colombia (variety CMC40) at 18–32°C and 60–95 per cent r.h.

Phenacoccus herreni was obtained from the Centro Internacional de Agricultura Tropical (CIAT), Colombia, in 1990 and reared on cassava at 22–30°C. A thelytokous *A. diversicornis* strain from Colombia (termed TD) was also obtained from CIAT, in 1991. It was reared on cassava containing mainly third and fourth instar *P. herreni* at 24–26°C and 55–65 per cent r.h.

Phenacoccus manihoti, obtained from Senegal and Nigeria via the International Institute of Tropical Agriculture (IITA), Benin, in 1985, was reared on cassava at 19–25°C. An arrhenotokous *A. diversicornis* strain (termed AD) originated from material collected between 1982 and 1986 in south-western Brazil (Löhr *et al.*, 1990) and was reared on cassava plants containing mainly third and fourth instar *P. manihoti*, at 24–30°C and 40–80 per cent r.h.

Experiments were carried out at 25 ± 0.5°C (unless stated otherwise) and 60 per cent r.h.

Check for reproductive mode of cultures

In order to check if our AD culture consisted of arrhenotokous females only, 36 virgin AD females

were confined with a *P. manihoti*-infested cassava plant. Their offspring should all be male. We obtained 144 males and no female offspring, and concluded that our AD culture was entirely arrhenotokous. A similar test was performed with the TD culture. The 120 TD individuals with which the culture was started and their F₁ offspring were female and we therefore concluded that the TD culture was entirely thelytokous.

Oviposition and rearing

Third and early fourth instar mealybugs (*P. herreni* or *P. manihoti*) were placed on a disc of cassava leaf that was placed in a petri dish upside down. A parasitoid was placed on the patch after the mealybugs had settled. Parasitized mealybugs were carefully removed with a small paint brush and placed on the lower side of the leaf of a cassava plant in small gauze cages. After mummification, mealybugs were taken from the leaf cages and placed individually in glass tubes. Emerged parasitoids were fed honey, either with or without antibiotic. Parasitoid emergence was checked daily and parasitoid survival (i.e. survival from oviposition to emergence) was calculated. Mealybugs that disappeared from the leaf cages and mealybugs that had dried out were not included.

Antibiotic and temperature treatment

We investigated whether thelytokous females could be cured to produce sons by feeding TD females honey mixed with antibiotics or rearing parasitized mealybugs at high temperature. Cured individuals could be used to determine if TD and AD could be crossed to produce fertile female offspring, which would give a clue as to whether arrhenotokous and thelytokous *A. diversicornis* belong to the same species.

Three antibiotics (tetracycline, rifampicin (rifadin) or sulphadiazine) were mixed with honey in concentrations of 1 per cent (1 mg antibiotic dissolved in 99 mg honey) or 5 per cent (5 mg antibiotic in 95 mg honey). TD mummies were placed individually in glass tubes. Directly after their emergence, these TD females, which form the parental (P) generation, received one type of antibiotic in one concentration during two days. Thereafter, they received pure honey. Freshly emerged F₁ (first offspring generation), F₂ and F₃ females either received honey mixed with antibiotic, or pure honey during their entire life. All females were allowed to oviposit in two mealybugs (always *P. herreni*) per day, starting on

day 3 of their lives. All parasitized mealybugs were reared out in leaf cages.

For the temperature treatment, TD mummies were placed individually in glass tubes. Emerged females received honey. They were allowed to oviposit in two *P. herreni* per day, starting on day 3 of their lives. Parasitized mealybugs were reared out at 37 or at 33°C.

Crosses; mating and insemination

AD females that had been isolated as mummies to ensure that they were virgin were allowed to mate with TD males. In order to obtain inseminated females, one female and one male were confined in a glass tube for one or two days. The sex of the offspring allowed us to distinguish between successful and unsuccessful crosses. The presence of female offspring would indicate that the TD sperm was used by the AD female; F₁ females are hybrids. F₁ males are not hybrid but AD. Epistatic interactions between genes in the parental strains may be disrupted during meiosis in hybrids, causing them to be sterile. The presence of epistatic interactions between the parental genes was investigated by checking the fertility of virgin hybrid F₁ females.

A failure to cross may result from a failure to inseminate (pre-mating barrier, no sperm transmission) or from a failure to use the sperm. We dissected the females and recorded the presence or absence of sperm in the spermatheca in order to distinguish between these possibilities.

Results

Male production

Treatment with antibiotics TD females that were fed the antibiotics tetracycline and rifampicin produced male offspring within two generations, and one female produced a son in the F₁ (Table 1). Note that the P females were fed antibiotics for only two days. Thelytokous *A. diversicornis* can thus be cured with antibiotics, leading to the conclusion that the asexuality is caused by microorganisms. F₁ TD females produced sons without being fed antibiotics themselves; thus, they were (at least partly—some still produced sons and daughters) cured because they laid eggs on which the microorganisms did not act. We cannot exclude the presence of the microorganisms; their numbers were at least lowered. Sulphadiazine did not result in male production in two generations and is therefore considered less suitable for obtaining TD males. There were no major differ-

Table 1 The effect of antibiotic and temperature treatment on offspring sex and survival (egg to emergence) of *Apoanagyrus diversicornis*

Treatment	Generation	Mother treated				Mother not treated			
		No. of mothers	No. of parasitized hosts	Per cent survival†	No. of ♂♂/ no. of offspring	No. of mothers	No. of parasitized hosts	Per cent survival†	No. of ♂♂/ no. of offspring
Control	F ₁					40	144	87.5	0/126
T 1 per cent	F ₁	7	25	60.0**	1/15				
	F ₂	7	46	60.9**	27/28	2	6	100	4/6
	F ₃					1	7	57.1	4/4
T 5 per cent	F ₁	4	11	54.6*	0/6				
	F ₂	1	2	50.0	1/1				
R 1 per cent	F ₁	7	10	80.0	0/8				
	F ₂	3	15	53.3**	8/8	1	9	100	9/9
R 5 per cent	F ₁	2	4	50.0	0/2				
S 1 per cent	F ₁	4	8	87.5	0/7				
	F ₂	3	58	86.2	0/50				
S 5 per cent	F ₁	2	5	80.0	0/4				
	F ₂	2	40	80.0	0/32				
		Parasitized hosts treated				Parasitized hosts not treated			
Temp. 37°C	F ₁	13	21	0.0**	—				
Temp. 33°C	F ₁	5	17	88.2	0/15				
	F ₂	4	19	57.9**	9/11	3	5	40.0*	2/2
	F ₃					1	8	62.5	5/5

All parasitoids were TD and all hosts were *Phenacoccus herreni*. Except for the controls, untreated wasps had a treated mother.

T, tetracycline; R, rifampicin; S, sulphadiazine; Temp., temperature at which offspring were reared out.

†Tested for difference from the survival of the control (χ^2 -test, * $P \leq 0.05$, ** $P \leq 0.005$).

ences between the results obtained with 1 per cent and 5 per cent concentrations of any of the antibiotics (Table 1).

Temperature treatment At 37°C, not a single parasitoid out of 21 parasitized *P. herreni* survived. This temperature was apparently too high to rear out *A. diversicornis*. We therefore switched to using a temperature of 33°C. All F₁ offspring were female. A proportion of the F₂ was male, both when reared at high and at normal temperatures, so their mothers were at least partly cured (Table 1).

Survival

We compared egg-to-adult offspring survival after treatment with survival of the controls, i.e. untreated TD. *Phenacoccus herreni* was the host species in all cases. Different treatments had different effects on offspring survival (Table 1).

Antibiotics Treatment of mothers with tetracycline resulted in a lower offspring survival, both in 1 per cent and 5 per cent concentrations. The rifampicin 1 per cent treatment lowered the offspring survival only if there were males among those offspring. Sulphadiazine treatment of mothers had no effect on offspring survival and it also did not result in male production.

Temperature A temperature of 37°C was lethal to the developing *A. diversicornis*. A temperature of 33°C had no effect on survival. Offspring survival was lower when males appeared among them, both when reared at 33 and at 25°C.

Mating ability

TD females clearly mated less often than AD females (Table 2). There were no differences in mating ability between TD and AD males: males of

both strains inseminated 100 per cent of the AD females and hardly any of the TD females (Table 2). Treating females or their mothers with antibiotics did not significantly affect their mating ability. A small negative effect of antibiotics cannot be excluded as matings were only obtained with untreated females. The sample size may be too small to ensure sufficient power to detect a possible effect.

Twenty-minute observations of *A. diversicornis* pairs directly after placing them together showed that males of both strains were active in courting all females. TD and AD males tried to approach TD

females as they did with AD females. TD females, however, often moved away from the male, whereas AD females allowed males to approach them. Within the observation period mating with an AD female was observed on several occasions, whereas no TD females were observed to mate.

Crosses

Seven of the 11 AD females that were inseminated by TD males (Table 3) produced daughters. Female offspring result from fertilized eggs and thus the F_1 females were a cross between the two strains. These hybrids will be denoted by ATD. The males in the F_1 resulted from unfertilized eggs of the AD females so they were AD. The survival from egg to adult of AD males in *P. manihoti* and *P. herreni* is known from separate experiments (Table 4), and allowed us to deduce the number of male and female eggs laid (i.e. the primary sex ratio). We calculated the survival probability of ATD F_1 females on the basis of the deduced primary sex ratio and the result is given in Table 4.

Three F_1 females were allowed to oviposit and so produce F_2 offspring (Table 3). The F_2 males resulted from unfertilized eggs of the ATD females and they were therefore ATD. The last line in Table 3 enabled the calculation of male ATD offspring survival in *P. manihoti* and *P. herreni*. ATD survival from egg to adult turned out to be equal for males and females (Table 4).

The F_1 hybrids were found to be fully fertile. The survival of hybrid F_2 was equal to the survival of hybrid F_1 individuals. This was true for hybrids reared from both mealybug species (Table 4). Moreover, F_2 hybrid survival in *P. herreni* was as high as the survival of both parental strains in this mealybug, although sample sizes are small. There were no indications of F_2 hybrid breakdown. It is concluded

Table 2 The proportion of female *Apoanagyrus diversicornis* that was inseminated after having been confined with a male for 1 or 2 days (number of pairs tested is indicated in parentheses)

♀	♂AD	♂TD
AD untreated	1.00 ^{AA} (18)	1.00 ^{AC} (8)
TD overall	0.06 ^{BB} (32)	0.00 ^{BD} (13)
untreated	0.10 (20)	0.00 (5)
treated	0.00 (12)	0.00 (8)
ATD untreated		0.33 ^{CD} (3)

Entries with the same letter are not significantly different from each other [Fisher's exact probability test, two-sided, $\alpha = 0.05$; small letters for tests between males (tested within rows only), capitals for tests between females (tested within columns only)]. As data are used more than once, the test statistics are dependent on each other. Hence, *P*-values have to be combined and dependencies must be taken into account. Therefore the improved Bonferroni method was applied. This method, which is explained in detail in Haccou & Meelis (1992, p. 248) and based on the original papers by Simes (1986) and Hochberg (1988), ensures that the overall α is at most 5 per cent. Treated: with antibiotics.

Table 3 The result of crosses between *Apoanagyrus diversicornis* strains

Cross	<i>n</i>	<i>P. herreni</i>			<i>P. manihoti</i>		
		No. of parasitized hosts	No. of ♂ offspring	No. of ♀ offspring	No. of parasitized hosts	No. of ♂ offspring	No. of ♀ offspring
AD♀ × TD♂	11	19	5	12	32	12	5
ATD (F_1)♀ × TD♂	1	0	—	—	10	1	0
ATD (F_1)♀	2	4	3	—	24	5	—

Insemination was confirmed by dissection of the females after oviposition. Because of a shortage of *Phenacoccus herreni* in the culture, *P. manihoti* was also used as a host. Survival of TD and ATD in this host species is, however, lower than in *P. herreni* (see Table 4). *n*, no. of female parasitoids.

that AD and TD *A. diversicornis* can be crossed to produce viable F₁ and F₂ offspring.

ATD survival in *P. manihoti* was about two-thirds less than in *P. herreni*, and intermediate between the survival of the parental strains (Table 4). ATD individuals inherited the low survival probability in *P. manihoti* from the TD parent: TD have a survival of only 0.045 in *P. manihoti* (Table 4). The F₂ survival of the hybrids might seem to be exceptionally low (Table 3), but this is caused by rearing them out in *P. manihoti* and it is not an indication of F₂ hybrid breakdown, as shown by high survival in *P. herreni*.

Discussion

Male production was induced by feeding thelytokous females the antibiotics tetracycline or rifampicin, and by rearing parasitized mealybugs at 33°C. This shows that asexuality in *A. diversicornis* was caused by microorganisms. PCR amplification of 16S ribosomal DNA from TD females (J.H. Werren, unpublished results) showed that the microorganism with which these are infected is, as was expected, a member of the genus *Wolbachia*. No *Wolbachia* ribosomal DNA could be found in AD females. This indicates that the arrhenotokous strain of *A. diversicornis* is indeed free of *Wolbachia*.

Crosses between male *A. diversicornis* from Colombia (TD) and female *A. diversicornis* from south-western Brazil (AD) were fully compatible. This suggests that, on an evolutionary time scale, the separation of AD and TD has occurred recently.

Stouthamer & Kazmer (1994) showed that *Wolba-*

chia act by preventing segregation of chromosomes during the first mitotic division (which takes place after oviposition). Antibiotics ingested by the *A. diversicornis* female or heat treatment of the eggs she laid did not have an effect on the sex of those eggs. Apparently, *Wolbachia* action on the F₁ eggs could not be prevented any more. This is analogous to the results of, for example, Gordh & Lacey (1976) and Laraichi (1978) found after heat treatment. Antibiotics administered to parental generation *A. diversicornis* reduced the numbers of *Wolbachia* in the F₁, because the F₂ also contained males, even if the F₁ were not treated. *Apoanagyrus* are synovigenic (i.e. they mature their eggs during their lives), but they carry some ripe eggs at eclosure. TD females might have produced a larger proportion of males in the F₁ if the antibiotics had been administered during their entire life instead of during two days only, or if the females had lived and oviposited longer. Antibiotics then might have prevented *Wolbachia* action on newly ripened eggs. The only F₁ male that appeared was laid by its mother on day 6 of her life, suggesting that it may have resulted from an egg that the mother ripened after she emerged. Females that were administered antibiotics usually did not live longer than 6 days.

Treatment with sulphadiazine during two generations did not lead to male production. The parasitoids given this treatment lived longer than parasitoids kept without food (pers. obs.), suggesting that the treated wasps have eaten the honey-sulphadiazine solution. The antibiotic may not have reached the microorganisms, have been ineffective, or taken more time to become effective. The longevity and fecundity of *Trichogramma pretiosum* were significantly lowered by tetracycline, but not by rifampicin or sulphamethoxazole (Stouthamer, 1990). Tetracycline also had the largest effect on *A. diversicornis*. It lowered the offspring survival from egg to adult. There was no effect of sulphamethoxazole. We found some mortality associated with the production of males by treated and untreated F₁ mothers in the rifampicin and temperature treatment. *Wolbachia* restore diploidy by gamete duplication, resulting in completely homozygous offspring (Stouthamer & Kazmer, 1994). Thus, deleterious mutations generally are expressed in *Wolbachia*-infected females. Deleterious mutations in genes encoding characters that are expressed in males only, however, are silent in females. Such mutations may be the cause of the mortality that was associated with male production.

Male behaviour and function were apparently normal in sons from cured thelytokous females.

Table 4 Survival of AD, TD and hybrid *Apoanagyrus diversicornis* in the hosts *Phenacoccus herreni* and *P. manihoti*

	AD	TD	ATD
<i>P. manihoti</i>	0.75 ^a (36)	0.045 ^b (44)	0.25 ^c (40)
	♀♀ + ♂♂ 0.74 (19)		♀♀ 0.31 (16)
	♂♂ 0.76 (17)		♂♂ 0.21 (24)
<i>P. herreni</i>	0.81 ^a (127)	0.88 ^a (144)	0.88 ^a (17)
			♀♀ 0.92 (13)
			♂♂ 0.75 (4)

Sample sizes are indicated in parentheses.

Entries with the same letter are not significantly different ($\alpha = 0.05$, χ^2 -test and Student's *t*-test).

The improved Bonferroni method (Haccou & Meelis, 1992) was applied.

Male and female survival were compared with each other for AD in *P. manihoti* and for ATD in both mealybug species; no differences were found ($\alpha = 0.05$).

Insemination by TD males occurred as frequently as insemination by AD males. The female sexual behaviour, however, was clearly affected: both asexual and cured TD females were much less often inseminated than AD females. A similar result was obtained by Rössler & DeBach (1972, 1973) for the aphelinid *Aphytis mytilaspidis* and by Stouthamer *et al.* (1990b) for strains of *Trichogramma pretiosum*. Carson *et al.* (1982) found a rapid decay of female sexual behaviour in a parthenogenetic strain of *Drosophila mercatorum*. Our data suggest an interesting difference between genes coding for male and female sexual behaviour, in the speed with which deleterious mutations accumulate in thelytokous females. Being not expressed in TD, the male sexual behaviour is expected to degenerate as a neutral character, whereas female sexual behaviour is expressed and may be selected against if there is a cost of maintaining sexual behaviour in thelytokous females. This difference between female traits (expressed and possibly selected against) and male traits (not expressed in asexuals) may lead to a higher rate of decay of female than of male sexual behaviour in an asexual population. This may explain the results of Carson *et al.* (1982).

Attempts to establish a sexually reproducing *A. diversicornis* strain from entirely thelytokous material failed because we could not obtain matings between asexual or cured TD females and TD males. This is analogous to the result of Zchori-Fein *et al.* (1992): 'cured' males of *Encarsia formosa* failed to inseminate asexual or cured conspecific females. An entirely sexually reproducing TD line would have made it possible to investigate whether the sex allocation behaviour of a strain had changed after a period of asexuality, by comparing it with the sex allocation of the AD strain. AD males occasionally inseminated TD females, but no genetic markers were available in *A. diversicornis* to investigate whether inseminated TD females use the sperm of AD males. The mating frequency of the ATD females appeared to be intermediate between that of the parental strains.

At least seven of the 11 AD females that were inseminated by TD males successfully used the sperm. The other four females may have laid female eggs as well, but the low survival of the ATD hybrids in *P. manihoti* caused the majority of the offspring to die. The hybrids only survived well in *P. herreni*. (At the time of the crossing experiments, there was a shortage of *P. herreni* in the culture, hence the extensive use of *P. manihoti* as a host.) Our results show that in *A. diversicornis*, offspring survival probability is not a maternally inherited factor but (like mating

frequency) appears to be intermediate between the survival probability of the maternal and paternal strain.

TD occur on *P. herreni* in Colombia, and AD have been found on *P. manihoti* in central South America. The low survival of TD in *P. manihoti* may be because of the absence of this host species from the parasitoid's native area (Commonwealth Institute of Biological Control, unpublished data). AD survive well in *P. manihoti* and in *P. herreni* and both host species occur in central South America (Yaseen, 1981; Löhr *et al.*, 1990). Gene flow between AD and TD is unlikely because, as far as we know, the populations are geographically isolated. If parasitoids were to migrate, gene flow from AD to TD would still be unlikely because TD females are rarely inseminated by AD males. TD males may occasionally occur because of high temperatures. If TD individuals migrated to central South America, genetic material of the two strains may be mixed because TD males cross with AD females. It is very unlikely that this would lead to the *Wolbachia* infection being transferred to AD females as sperm usually do not carry *Wolbachia*. Horizontal transmission of *Wolbachia* via the host (i.e. by superparasitism) may be possible. The lack of congruence between the phylogeny of *Wolbachia* and their insect hosts suggests that horizontal transfer of *Wolbachia* has occurred frequently (O'Neill *et al.*, 1992; Rousset *et al.*, 1992; Werren *et al.*, 1995).

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