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Biological control of cactus weeds: implications of hybridization between control agent biotypes

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Summary

1. Results of recent research on *Dactylopius opuntiae*, a biological control agent for cactus weeds (*Opuntia* spp.) in South Africa and elsewhere, challenge the maxim that genetic diversity of agents necessarily enhances the chances of success in biological weed control.

2. Two biotypes of *D. opuntiae*, each specific to a different *Opuntia* species, interbred freely, at least under insectary conditions. We therefore carried out cross-breeding experiments to determine the viability and host-preferences of progeny produced by these crosses.

3. Unlike their parents, F_1 hybrids were not species-specific, developing equally well on either of the parental hosts, *Opuntia ficus-indica* and *Opuntia stricta*. The situation was more complex in F_2 back-crosses between hybrids and in crosses between parent strains and hybrids because male cochineal insects contributed only maternally inherited genes to their progeny, due to their unusual haploid-diploid (lecanoid) mechanism of sex determination. Some F_2 combinations produced cohorts of progeny that were either entirely true-bred (i.e. host-specific) or entirely hybrid (i.e. not host-specific) genotypes, while other combinations produced groups of siblings with some individuals (theoretically half) that were true-bred genotypes and the balance were hybrid genotypes.

4. The lack of host-specificity of hybrids should enhance overall biological control of the target species directly, because hybrids attack both host-plants, and indirectly, because hybrid nymphs have greater chances of finding a suitable host-during passive dispersal. However, this advantage will be negated when F_2 crosses produce host-specific nymphs on host-plants that are incompatible for their survival.

5. These findings show that only pure strains of *D. opuntiae* should be released in monocultures of the target weeds. More generally, they caution that the possible consequences of mixing genotypes of a biological control agent species should be investigated before different provenances are amalgamated to enhance genetic diversity.

Key-words: Cactaceae, *Dactylopius opuntiae*, genetic diversity, host-specificity, *Opuntia*.

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Introduction

Cochineal insects (Homoptera: Dactylopiidae) have featured for many years in the biological control of weedy *Opuntia* species (Cactaceae) around the world, sometimes with exceptional positive results (Moran & Zimmermann 1984; Julien & Griffiths 1998). Among the most widely used and successful species has been *Dactylopius opuntiae* (Cockerell), which has contributed extensively to the control of *Opuntia stricta* (Haw.) in Australia (Dodd 1940; Hosking, Sullivan & Welsby 1994) and *Opuntia ficus-indica* (L.) Miller in South Africa (Pettey 1948), as well as other weedy *Opuntia* species elsewhere (Moran & Zimmermann 1984; Julien & Griffiths 1998). *Opuntia ficus-indica* in particular, and *O. stricta* to a lesser extent, occurs over a wide range within South Africa (Henderson 2001); the two species occur together in close proximity in several areas of the country (Fig. 1). It was thus puzzling that, for many years, *D. opuntiae* failed to utilize *O. stricta* successfully as a host in South Africa, even though the insect had been abundant throughout the country for almost 60 years on *O. ficus-indica*.

The dilemma was resolved recently when it was confirmed that *D. opuntiae* comprised at least two distinct biotypes, each specifically associated with a particular

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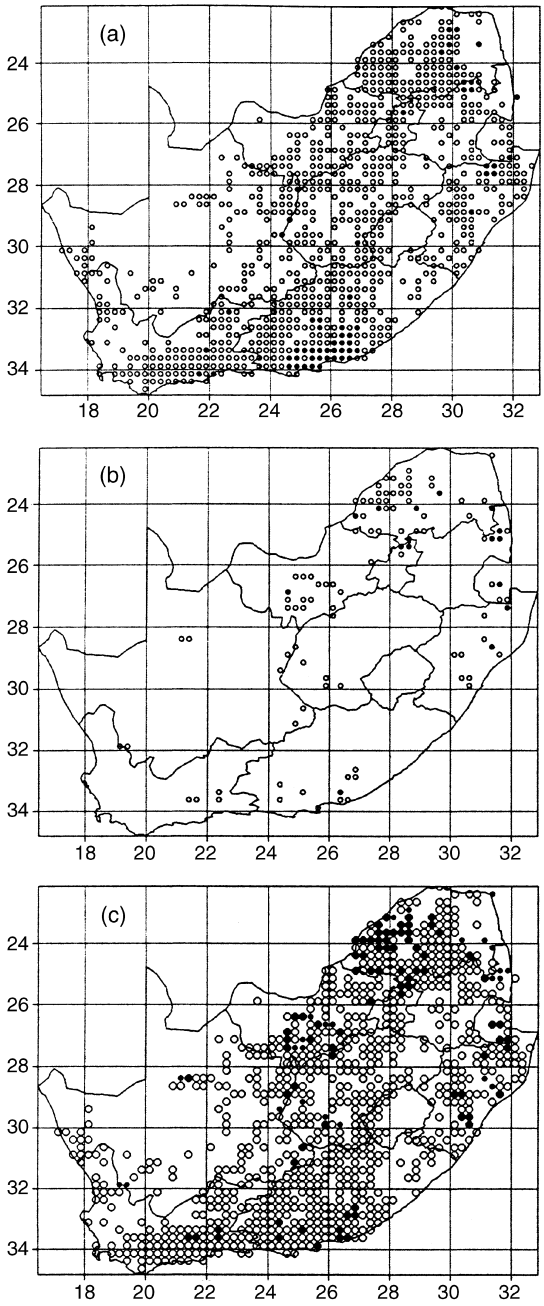

Fig. 1. Maps showing distributions of (a) *Opuntia ficus-indica* and (b) *Opuntia stricta* (dots represent regions where extensive dense clumps of plants occur, while circles show regions where plants are sparse), together with an overlay (c) to show where the two species co-occur (dots surrounded by circles) or occur in isolation (circles for *O. ficus-indica*, dots for *O. stricta*) (L. Henderson, unpublished data). Grid lines indicate prime degrees of longitude east and latitude south.

Opuntia species (Githure, Zimmermann & Hoffmann 1999; Volchansky, Hoffmann & Zimmermann 1999). Experiments showed that the biotype of *D. opuntiae* in South Africa, referred to as the 'ficus' biotype, favoured development on *O. ficus-indica* above *O. stricta*, while the one from Australia, referred to as the 'stricta' biotype, favoured development on *O. stricta* above *O. ficus-indica.* Scrutiny (J.H. Hoffmann, unpublished data) of reports compiled between 1920 and 1935 from the Alan Fletcher Research Station (now housed in Queensland State Archives, Brisbane, Australia) showed that researchers responsible for the introduction of *D. opuntiae* into Australia in 1921 and South Africa in 1938 were aware of differences in the host-specificity of *D. opuntiae* from different regions and host-plant species in the Americas. The reports also showed that considerable effort was made to keep each provenance separate during mass-rearing and release operations. As a result, the provenance of *D. opuntiae* that was originally released against *O. stricta* in Australia was distinct from that released against *O. ficus-indica* in South Africa, even though the latter was obtained via Australia (Dodd 1940; Pettey 1948). The recent release (1997) of the 'stricta' biotype in South Africa has resulted in *D. opuntiae* becoming established on *O. stricta*, with thriving populations inflicting considerable damage and mortality on the weed at many widespread localities (Hoffmann & Zimmermann 1999).

In an attempt to ascertain the taxonomic status of the two biotypes, cross-breeding experiments were undertaken to determine whether the two taxa might be considered separate genetical species. Besides being of fundamental interest, the result was considered to be of functional significance because, if the biotypes interbreed freely, the viability and specificity of hybrid progeny could influence the effectiveness of the insects as biological control agents of the two target weeds. If hybrids can only develop on one or other of the two *Opuntia* species, the usefulness of the insects for biological control of the other species might be diminished. Alternatively, if the hybrids have no developmental restrictions on either host, biological control of both target weed species could be enhanced because there would be a greater assemblage of suitable hosts available to the passively dispersed crawlers (= first-instar nymphs) in areas where the two plant species are sympatric.

This paper reports on the results of the cross-breeding experiments. As such, it differs from the earlier studies of Githure, Zimmermann & Hoffmann (1999) and Volchansky, Hoffmann & Zimmermann (1999), which reported on the host-specificity of the true-bred 'ficus' and 'stricta' biotypes of *D. opuntiae*.

Materials and methods

To determine the developmental success of different lineages of *D. opuntiae* on *O. stricta* and *O. ficus-indica*, different familial lines were produced and monitored, including F_0 true-bred strains, F_1 hybrids and F_2 crosses of hybrids and back-crosses of hybrids with true-bred strains in various combinations. The following convention is used to represent the genotypes of the familial lineages. The letters 'F' and 'S' represent the biotypes 'ficus' or 'stricta', respectively. Couplets of letters are used to show lineage, with two capital letters representing females and a capital letter together with

a lower-case letter representing males, so that 'FF' and 'Ff' depict true-bred 'ficus' biotype females and males, respectively, and 'SS' and 'Ss' depict true-bred 'stricta' biotype females and males, respectively. F_1 hybrids are depicted as either FS, for females, or Fs, for males, derived from 'ficus' females crossed with 'stricta' males, and SF, for females, or Sf, for males, derived from 'stricta' females crossed with 'ficus' males. The same convention is used for F_2 genotypes derived from different combinations of crosses and back-crosses, but pairs of couplets separated by a hyphen depict the lineage of each F_2 cross, giving the following combinations: FS-Ff, SF-Ff, FF-Fs, FF-Sf, FS-Ss, SF-Ss, SS-Fs, SS-Sf, FS-Fs, FS-Sf, SF-Fs and SF-Sf. In each combination of couplets the first pair represents the maternal lineage and the second the paternal lineage.

True-bred lineages of each biotype were maintained by ensuring that males only mated with females of the same biotype. To produce F_1 hybrids and F_2 crosses and back-crosses, male pupae, which are easily distinguishable (Moran & Cobby 1979), were removed from caged cladodes with *D. opuntiae* colonies of a particular genotype and introduced into cages with only females of another genotype. In this way two distinct F_1 lineages and 12 distinct F_2 lineages were produced (Table 1).

For each lineage, batches of females (for *n*-values see Table 2) that had just started to produce eggs were removed from the cladodes on which they had developed. The females were dewaxed by winding the prolific wax coat that envelopes each female onto a fine spindle made from two pins. Each female was then

Table 1. Parent genotypes of *Dactylopius opuntiae* used in various combinations of cross-mating experiments over three generations and the resultant genotypes and phenotypes of the progeny expected from each cross. Reference to the corresponding figures in the text is shown for each cross

		Parent genotypes		Progeny genotypes			
Generation	Cross category	Female	Male	Female	Male	Progeny phenotypes	Figure
F_0		FF	Ff	FF	Ff	'Ficus'	2a
F_0		SS	Ss	SS	Ss	'Stricta'	2b
F_1	i	FF	Ss	FS	Fs	Hybrid	3a
F_1	i	SS	Ff	SF	Sf	Hybrid	3b
F_2	ii	FF	Fs	FF	Ff	'Ficus'	4a
F_2	ii	SS	Sf	SS	Ss	'Stricta'	4b
F_2	ii	FF	Sf	FS	Fs	Hybrid	4c
F_2	ii	SS	Fs	SF	Sf	Hybrid	4d
F_2	iii	FS = SF	Ss	FS + SS	Fs + Ss	'Stricta' + hybrid	5a
F_2	iii	FS = SF	Ff	FF + SF	Ff + Sf	'Ficus' + hybrid	5b
F_2	iv	FS = SF	Sf	FS + SS	Fs + Ss	'Stricta' + hybrid	6a
F_2	iv	FS = SF	Fs	FF + SF	Ff + Sf	'Ficus' + hybrid	6b

Table 2. Numbers and mass (mean ± SE) and mate success (%) of female progeny harvested from *Opuntia ficus-indica* (= *O. f-i.*) and *Opuntia stricta* for different lineages of *Dactylopius opuntiae* biotypes. *n* = number of parent crosses made for each lineage. ANOVA is for comparisons of mean values in columns

		Female harvested cladode^{-1} on		Female mass (mg) on		Female mate success on	
Lineage	*n*	*O. f-i.*	*O. stricta*	*O. f-i.*	*O. stricta*	*O. f-i.*	*O. stricta*
FF-Ff	20	13·8 ± 1·1	8·6 ± 1·0	10·4 ± 0·3	2·1 ± 0·2	90·5	16·9
SS-Ss	20	5·5 ± 1·1	13·4 ± 1·0	1·5 ± 0·1	13·3 ± 0·4	10·1	91·8
FF-Ss	20	15·4 ± 1·1	14·0 ± 1·0	9·9 ± 0·3	10·5 ± 0·4	84·4	79·6
SS-Ff	20	13 ± 1·1	14·6 ± 1·0	8·1 ± 0·3	10·2 ± 0·4	64·2	81·5
FF-Fs	19	20·6 ± 1·2	11·3 ± 1·0	6·5 ± 0·2	4·2 ± 0·3	79·6	53·3
SS-Sf	19	7·1 ± 1·2	12·0 ± 1·0	2·3 ± 0·2	12·4 ± 0·5	36·6	91·7
FF-Sf	24	15·6 ± 1·0	15·9 ± 0·9	7·0 ± 0·3	12·0 ± 0·4	77·0	91·4
SS-Fs	22	12·9 ± 1·1	15·9 ± 0·9	7·6 ± 0·4	10·4 ± 0·3	75·6	92·0
FS-Ss	24	11·5 ± 1·0	16·0 ± 0·8	4·2 ± 0·3	12·2 ± 0·4	42·8	77·2
SF-Ss	30	9·6 ± 0·9	16·0 ± 0·8	3·5 ± 0·2	12·3 ± 0·3	34·5	80·2
FS-Ff	29	17·4 ± 1·0	11·8 ± 1·0	7·8 ± 0·4	10·5 ± 0·3	81·4	71·4
SF-Ff	29	13·7 ± 0·9	14·1 ± 0·8	8·8 ± 0·3	7·1 ± 0·3	76·3	60·1
FS-Sf	45	12·0 ± 0·9	16·1 ± 0·8	6·9 ± 0·2	10·4 ± 0·3	59·8	80·0
SF-Sf	30	11·2 ± 1·2	16·0 ± 0·6	6·7 ± 0·3	11·3 ± 0·4	53·6	69·6
FS-Fs	30	13·2 ± 0·8	16·3 ± 0·8	7·6 ± 0·3	10·1 ± 0·3	64·9	80·8
SF-Fs	40	14·9 ± 0·6	14·9 ± 0·7	7·2 ± 0·2	9·1 ± 0·3	75·3	80·8
ANOVA		$F_{15,405} = 11·7$ $P < 0·00001$	$F_{15,405} = 5·80$ $P < 0·00001$	$F_{15,5451} = 55·8$ $P < 0·00001$	$F_{15,6082} = 47·6$ $P < 0·00001$		

placed in a container on her own and left to produce offspring. Sixty of the crawlers produced by each female were transferred with a paint brush onto cactus cladodes in two batches, so that 30 of the crawlers were placed on an *O. stricta* cladode and 30 were placed on an *O. ficus-indica* cladode. Thirty insects per 50 g of cladode ensures ample food supply (Hosking 1984; Sullivan 1990) and, in this case, all cladodes weighed more than 50 g.

The cladodes with insects were retained in a controlled environment room with fluorescent lights on a 12-h daylight cycle and a temperature regime of 28 ± 2 °C during 'daylight' and 24 ± 2 °C during 'night', to provide a suitable environment for the insects (Hosking 1984; Sullivan 1990; Guerra & Kosztarab 1992). The cladodes were harvested from the terminal sections of potted *O. stricta* and *O. ficus-indica* plants that were maintained in a greenhouse. Detached cladodes remain alive for several months without contact with the soil, and cochineal insects can be reared as effectively on isolated cladodes as on rooted plants (Moran & Cobby 1979; Moran, Gunn & Walter 1982; Hosking 1984; Sullivan 1990). Each cladode was suspended horizontally on the heads of four pins that had been inserted into a polystyrene block. This allowed crawlers to settle almost anywhere on the surface of the cladode and minimized avenues from which the crawlers could move off the cladodes.

The cladodes with each familial lineage of cochineal were retained in close proximity to allow the males to intermingle freely with and mate with the females. As soon as some females started producing offspring on a cladode, all the females were removed from that cladode. The females were dewaxed and weighed before being placed in separate containers. The number of females harvested from each cladode provided a measure of the proportion of crawlers that survived to maturity. Survival of males was not measured directly because their small size and secretive habits at all stages of the life cycle rendered their detection unreliable. Nevertheless there was evidence that sex ratios conformed to unity because for each lineage where crawlers were placed on compatible hosts (i.e. FF-Ff, FF-Ss, SS-Fs, FF-Fs, FF-Sf and SS-Fs on *O. ficus-indica*, and SS-Ss, FF-Ss, SS-Fs, SS-Sf, FF-Sf, and SS-Fs on *O. stricta*) the proportion of the original population of crawlers that matured as females never differed significantly from 0·5 (χ^2 = 3·55 and 1·26 for 9 degrees of freedom on *O. ficus-indica* and *O. stricta*, respectively). Over the next 3 weeks, inspections were made to determine which of the harvested females had produced offspring so that mate-success was ascertained. Mated females lay eggs regardless of their location, on or off the host-plant, while unmated females never lay viable eggs (Sullivan 1990; J.H. Hoffmann, unpublished data).

To compare the developmental success of different lineages on each host-plant, survival (number of mature females produced) and the average number of progeny produced per original crawler was used to calculate *R*, the 'net rate of increase' (Begon & Mortimer 1981), for each paired batch of siblings on both plant species using:

$$R = (f \times p)/30 \qquad \text{eqn 1}$$

where *f* is the number of fertile females (i.e. those that survived and produced progeny) and *p* is the average number of progeny produced per fertile female.

The relationship between body size and numbers of progeny produced was calculated from a regression of mass on fecundity derived from a sample of 48 females of known mass whose progeny were counted. The sample included 12 females from each of four lineages (FF-Sf; FS-Sf; SF-Fs; SS-Fs). A pooled regression was used because there were no statistically significant differences between any of the regressions for each lineage and a single regression for all lineages combined and because the relationship between mass and fecundity was close (R^2 = 0·9). The regression statistic used was:

$$P = 23{\cdot}8m - 2{\cdot}2 \qquad \text{eqn 2}$$

where *m* is the average mass (mg) of the fertile females.

The *R*-value for each batch of siblings from each female on *O. stricta* was plotted on a graph against the *R*-value for the corresponding batch on *O. ficus-indica*, and these were designated *R*'stricta' and *R*'ficus', respectively. Host-specific lineages had high *R*-values on one host-species and not the other, while non-specific lineages had equivalent *R*-values on both host-plant species.

Results

The results of the cross-breeding experiments need to be interpreted in the light of the unusual (lecanoid) chromosome systems found in *Dactylopius* species (Hughes-Schrader 1948; Brown 1959, 1977; Nur 1990). Paternally inherited chromosomes are heterochromatic in males and are lost entirely during spermatogenesis, so that only maternally inherited chromosomes are transmitted by males from one generation to the next (Brown 1969; Bull 1979). Females are fully diploid and pass both maternal and paternal chromosomes to their progeny.

The data used to calculate *R*-values are summarized in Table 2. The values showed that female mass and mate success (which were closely correlated, *r* = 0·92) contributed most to the differences in *R*-values on the two host-plant species, but survival (females harvested cladode^{-1}) was also variable. Regardless of genotype, survival, mean mass and mate success of *D. opuntiae* were lower on *O. ficus-indica* (*n* = 5467; mean mass ± SE = 7·3 ± 0·1 mg; mate success = 67·5%) than on *O. stricta* (*n* = 6098; mean mass ± SE = 10·1 ± 0·1 mg; mate success = 77·0%), indicating that in general *O. stricta* was a more favourable host-plant than *O.*

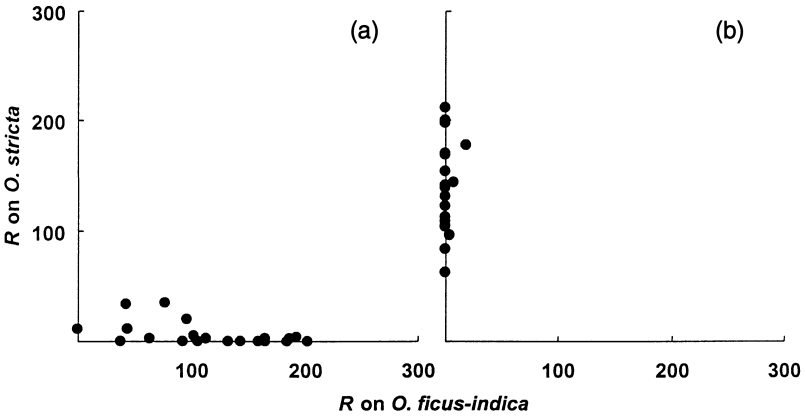

Fig. 2. *R*-values for true-bred progeny of 'ficus' and 'stricta' biotypes of *D. opuntiae.* (a) 'Ficus' females (FF) crossed with 'ficus' males (Ff); (b) 'stricta' females (SS) crossed with 'stricta' males (Ss).

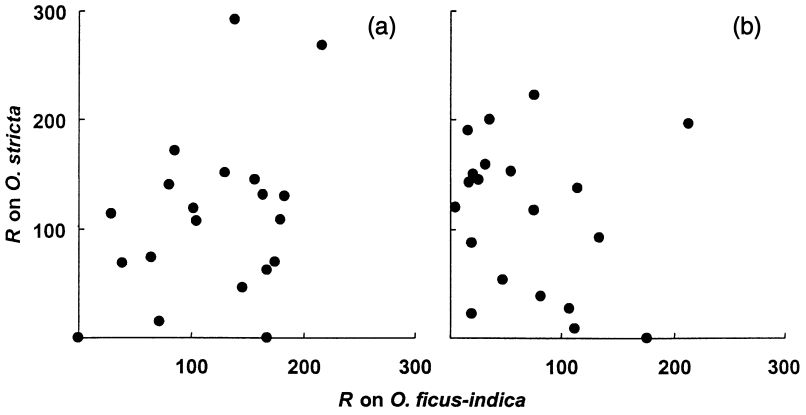

Fig. 3. *R*-values for F_1 progeny of crosses between 'ficus' and 'stricta' biotypes of *D. opuntiae.* (a) 'Ficus' females (FF) crossed with 'stricta' males (Ss); (b) 'stricta' females (SS) crossed with 'ficus' males (Ff).

ficus-indica for the insects (Githure, Zimmermann & Hoffmann 1999; Volchansky, Hoffmann & Zimmermann 1999).

R-values for the true-bred lineages of the 'ficus' and 'stricta' biotypes showed distinct differences between the two lines (Fig. 2). In Fig. 2a the points clustered close to the *x*-axis because the 'ficus' (FF, Ff) biotype of *D. opuntiae* developed significantly better on *O. ficus-indica* than on *O. stricta* (paired $t_{19} = 7{\cdot}48$, $P < 0{\cdot}00001$). In Fig. 2b the points clustered close to the *y*-axis because the stricta (SS, Ss) biotype of *D. opuntiae* developed significantly better on *O. stricta* than on *O. ficus-indica* (paired $t_{19} = 15{\cdot}2$, $P < 0{\cdot}00001$).

The possible combinations of F_1 crosses and F_2 crosses and back-crosses from the two biotypes of *D. opuntiae* fell into four main categories, which are shown in Table 1.

F_1 GENERATION CROSSES OF THE TWO ORIGINAL TRUE-BRED (F_0) BIOTYPES

In both cases (i.e. FF-Ss and SS-Ff) the *R*-values of the F_1 progeny fell more or less equidistantly between the two axes of the graph because hybrids of the two biotypes were not species-specific and developed equally well on either host-plant species (Fig. 3a,b) (paired $t_{19} = 0{\cdot}53$, $P = 0{\cdot}60$, and paired $t_{19} = 1{\cdot}99$, $P = 0{\cdot}06$, for *R*'stricta' compared with *R*'ficus' in Fig. 3a,b, respectively). The progeny of these initial crosses provided the hybrid genotypes used in the F_2 crosses and back-crosses.

F_1 HYBRID MALES BACK-CROSSED WITH F_0 PARENTAL TRUE-BRED BIOTYPES

This enabled four possible combinations of crosses, two of which resulted in females that only produced progeny with *R*-values that closely resembled (but with some significant differences due to greater spread in *R*-values) those of the host-specific parental F_0 biotypes (Fig. 4a,b) ($t_{37} = 3{\cdot}36$, $P = 0{\cdot}0018$ for *R*'stricta', and $t_{37} = 1{\cdot}38$, $P = 0{\cdot}17$ for *R*'ficus', in Fig. 4a compared with Fig. 2a, and $t_{37} = 1{\cdot}40$, $P = 0{\cdot}17$ for *R*'stricta', and $t_{37} = 3{\cdot}11$, $P = 0{\cdot}004$ for *R*'ficus', in Fig. 4b compared with Fig. 2b). The other two crosses resulted in females that only produced progeny with *R*-values indicative of hybrid genotypes that showed no host-specificity (Fig. 4c,d), and the distribution patterns of *R*-values equated with those in Fig. 3a,b ($F_{3,82} = 1{\cdot}86$, $P = 0{\cdot}14$ for *R*'stricta' values and $F_{3,82} = 3{\cdot}96$, $P = 0{\cdot}01$ for *R*'ficus' values). The significance in *R*'ficus' resulted from the FF-Ss lineage (Fig. 3a) having a significantly higher

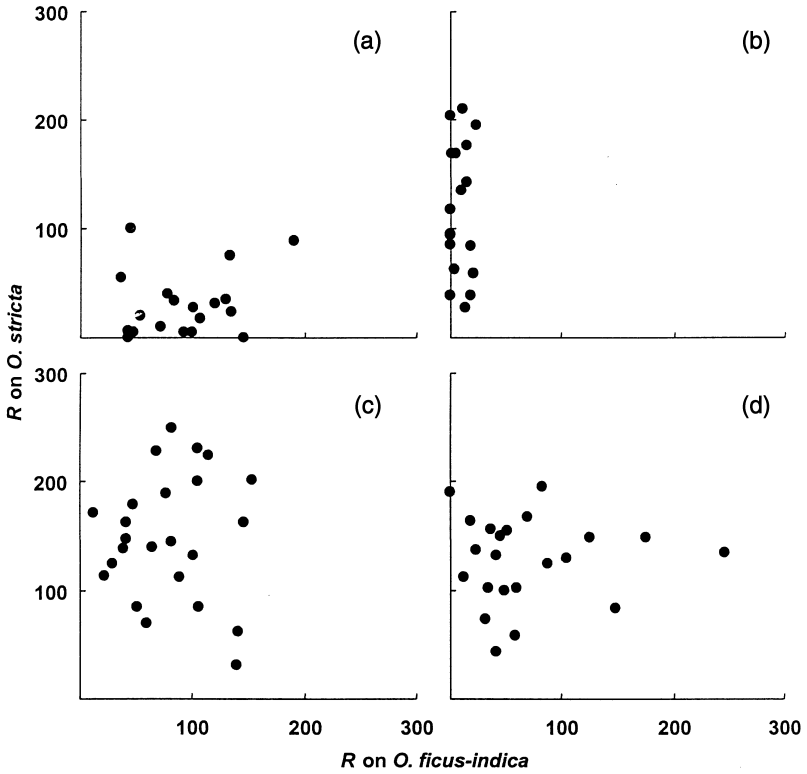

Fig. 4. *R*-values for F_2 progeny of back-crosses between true-bred female biotypes and hybrid males of *D. opuntiae*. (a) FF females crossed with Fs males; (b) SS females crossed with Sf males; (c) FF females crossed with Sf males; (d) SS females crossed with Fs males.

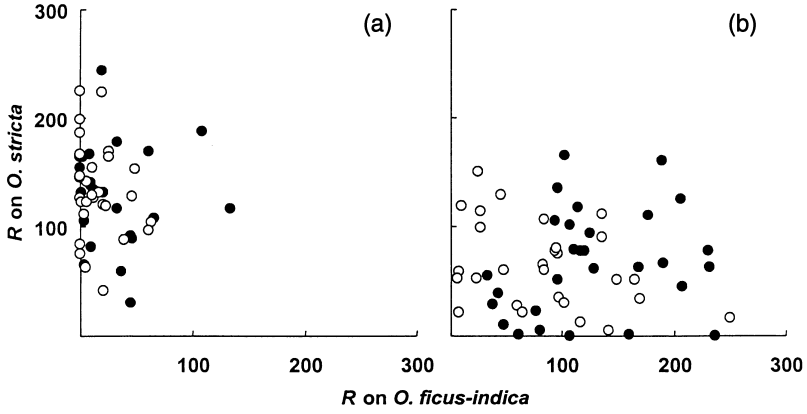

Fig. 5. *R*-values for F_2 progeny of back-crosses between hybrid females and true-bred males of *D. opuntiae*. (a) FS females (●) and SF females (○) crossed with Ss males; (b) FS females (●) and SF females (○) crossed with Ff males.

value than the SS-Ff (Fig. 3b) and SS-Fs (Fig. 4d) lineages.

F_1 HYBRID FEMALES CROSSED WITH F_0 MALES

In these crosses the mixture of genotypes was the same (Kolmogorov–Smirnoff test) regardless of whether the F_1 females were FS or SF (open circles compared with closed circles in Fig. 5a,b) and the data were pooled. The progeny of each female in these crosses was expected to segregate into two distinct genotypes. Approximately half of the siblings would be hybrid genotypes (with no host-specificity), while the other half would be true-bred (host-specific) genotypes (Table 1). As there was no way of distinguishing the genotypes of the individuals in batches of siblings, the pooled *R*-values were expected to be mediated by the combined contribution of the 'true-bred' and 'hybrid' individuals, and *R* was expected to be intermediate between the true-bred values (Fig. 2a,b) and the hybrid values (Fig. 3a,b). A series of *t*-tests showed that, as expected, the *R*-values were consistently intermediate in value, supporting the supposition that these groups of siblings consisted of both genotypes (Table 3).

Table 3. Group *t*-test statistics of *R*-values for groups of back-crossed individuals (Figs 5 and 6) compared with true-bred (Fig. 2a or 2b) or hybrid (Fig. 3a,b combined) genotypes

Comparison	*t* (degrees of freedom)	*P*
R'ficus', Fig. 5a with Fig. 2b	3·22 (72)	0·0018
R'ficus', Fig. 5a with Fig. 3a,b	7·55 (92)	< 0·0001
R'stricta', Fig. 5b with Fig. 2a	5·87 (76)	< 0·0001
R'stricta', Fig. 5b with Fig. 3a,b	4·06 (96)	0·0001
R'ficus', Fig. 6a with Fig. 2b	3·63 (93)	0·0005
R'ficus', Fig. 6a with Fig. 3a,b	3·56 (113)	0·0005
R'stricta', Fig. 6b with Fig. 2a	8·53 (88)	< 0·0001
R'stricta', Fig. 6b with Fig. 3a,b	0·40 (108)	0·6922

F_1 HYBRID FEMALES CROSSED WITH F_1 HYBRID MALES

The mixture of genotypes in the progeny of these crosses was the same (Kolmogorov–Smirnoff test) regardless of whether the F_1 females were FS or SF (open circles compared with closed circles in Fig. 6a,b) and the data were pooled. These crosses were also expected to produce a mixture of 'true-bred' and 'hybrid' genotypes in the siblings from each female (Table 1). A series of *t*-tests supported this expectation with one exception (Table 3). The *R*'stricta' values in Fig. 6b did not differ significantly from those of the hybrid genotypes. Possible reasons for this anomaly are raised in the Discussion.

Discussion

The results of this study mirror those described by Brown (1969) for cross-breeding experiments with wild-type and 'salmon-eye colour' phenotypes of the common mealybug *Planococcus citri* (Risso), where it was found that paternally inherited traits were not passed on to the next generation.

In general there is considerable scatter in the distribution of data points in the graphs depicted in this study, and thus some results deviate from expected. This is particularly noticeable in Fig. 6b, where more points (= 'ficus' genotypes) were expected to have fallen closer to the *x*-axis of the graph. These anomalies arose through the wide range of factors that influenced survival and development of the insects under the experimental conditions, including (i) nutritional condition of the cladodes; (ii) skewed sex-ratios in the cohorts of crawlers placed on the cladodes (James 1937; Nelson-Rees 1960); (iii) a proportion of the crawlers inadvertently falling from the cladodes prior to settling; (iv) *O. stricta* generally being a more benign host for the insects than *O. ficus-indica* (Githure, Zimmermann & Hoffmann 1999; Volchansky, Hoffmann & Zimmermann 1999); (v) relative isolation of settled females (those that settled close to one another tended to be smaller at maturity than those that were remote from others); (vi) feeding position of the insects on the cladodes (generally females on the lower surface of the cladode were larger at maturity than those on the upper surface); (vii) failure of some females to mate; and (viii) mixing of genes through chromosomal crossing-over during oogenesis in females, although this does not occur during spermatogenesis in males (White 1973). Nevertheless, even with three statistically significant exceptions (Figs 4a,b and 6b) the distribution of data points in each graph closely approximated a pattern that would be expected from the crosses shown in Table 1.

The fact that the two biotypes of *D. opuntiae* interbreed freely, at least under controlled insectary conditions, may have considerable implications for the biological control of *O. ficus-indica* and *O. stricta*. Provided there are no barriers to interbreeding between the 'stricta' and 'ficus' biotypes of *D. opuntiae* in the field, the loss of host-specificity in hybrid progeny could enhance biological control of *O. ficus-indica* and *O. stricta* in areas where the two weed species occur together. As interplant dispersal of cochineal insects is passive, the probability that a crawler will reach a new host increases proportionately with the density of suitable host-plants within the dispersal range of the

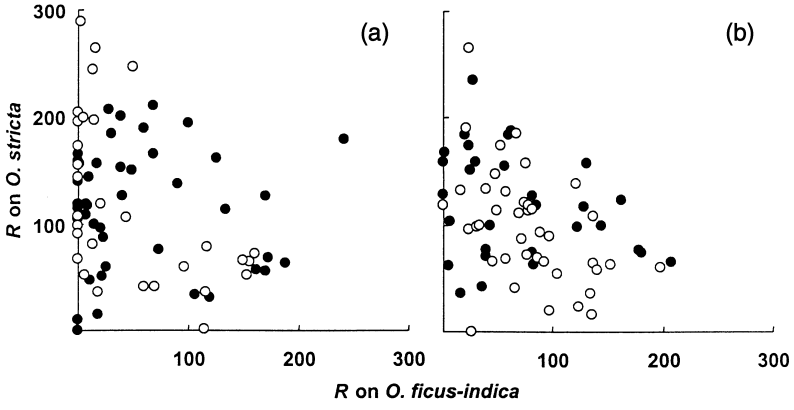

Fig. 6. *R*-values for F_2 progeny of crosses between hybrid females and hybrid males of *D. opuntiae*. (a) FS females (●) and SF females (○) crossed with Sf males; (b) FS females (●) and SF females (○) crossed with Fs males.

crawlers (Moran, Gunn & Walter 1982). Consequently, in areas with both *O. ficus-indica* and *O. stricta*, survival of hybrid crawlers will be higher than that of either of the host-specific parental biotypes because the chances of a non-specific crawler landing on a suitable host will increase relative to the combined abundance of the two host-plant species, either of which will be a suitable host. Higher levels of survival could in turn elevate the overall abundance of the populations of *D. opuntiae* and augment the levels of damage inflicted on the two target weed species.

On the other hand, the reversion to host-specific genotypes that results from F_2 crosses could be detrimental in cases where these genotypes are born on a host-plant species that is not suitable for survival and development (i.e. 'ficus' genotypes born on *O. stricta* or, conversely, 'stricta' genotypes born on *O. ficus-indica*). When this happens the incorrectly situated crawlers will need to disperse to a suitable host. Those that do not disperse will develop poorly and will contribute very little, if at all, to the next generation. Of those that do disperse, many will perish without encountering the correct host. Either way, survival and development of these crawlers will diminish, which in turn will curb the rate of increase of the populations of *D. opuntiae* to the detriment of the biological control programme.

Populations of the insects are being monitored in areas where both biotypes of *D. opuntiae* have been introduced and where both *Opuntia* species grow together (Fig. 1) to determine whether or not hybridization is occurring naturally. The consequences of any hybridization may be mediated by the relative abundance and density of the two host-plant species in any particular situation and this will also need to be monitored. The findings of this study should caution biocontrol practitioners in general not to mix hastily different provenances of a biological control agent until the consequences of such mixing have been thoroughly investigated and resolved.

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