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Pest fruit fly (Diptera: Tephritidae) in northwestern Australia: one species or two?

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Abstract

Since 1985, a new and serious fruit fly pest has been reported in northwestern Australia. It has been unclear whether this pest was the supposedly benign endemic species, *Bactrocera aquilonis*, or a recent introduction of the morphologically near-identical Queensland fruit fly, *B. tryoni*. *B. tryoni* is a major pest throughout eastern Australia but is isolated from the northwest region by an arid zone. In the present study, we sought to clarify the species status of these new pests using an extensive DNA microsatellite survey across the entire northwest region of Australia. Population differentiation tests and clustering analyses revealed a high degree of homogeneity within the northwest samples, suggesting that just one species is present in the region. That northwestern population showed minimal genetic differentiation from *B. tryoni* from Queensland ($F_{ST} = 0.015$). Since 2000, new outbreaks of this pest fruit fly have occurred to the west of the region, and clustering analysis suggested recurrent migration from the northwest region rather than Queensland. Mitochondrial DNA sequencing also showed no evidence for the existence of a distinct species in the northwest region. We conclude that the new pest fruit fly in the northwest is the endemic population of *B. aquilonis* but that there is no genetic evidence supporting the separation of *B. aquilonis* and *B. tryoni* as distinct species.

Keywords: Fruit fly, *Bactrocera aquilonis*, *Bactrocera tryoni*, population differentiation, allopatry, tephritid

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Introduction

True fruit flies (Diptera: Tephritidae) are serious pests of horticulture worldwide, due to their broad larval host range, cosmopolitan distribution and high invasive capacity (McPheron & Steck, 1996; Malacrida *et al*., 2007). The major fruit fly pest in Australia is the Queensland fruit fly, *Bactrocera tryoni* (Froggatt). Native to Queensland, this species spread south in the 1860s (Drew, 1989) and is now found along the entire east coast of Australia (Meats, 1981) where it infests almost all horticultural crops (Drew, 1978). Genetic structuring in these east coast populations have been studied in Yu *et al*. (2001), Meats *et al*. (2003) and Sved *et al*. (2003).

In addition to the east coast populations, Australia has a second endemic fruit fly region, the northwestern region comprising the Northern Territory and Western Australia (fig. 1). The east and northwest regions are separated by an arid zone straddling the border between the Northern Territory and Queensland (Drew, 1989).

Until recently, the northwest region was considered to be relatively free from serious fruit fly pests. The main species described in the region, *B. aquilonis* (May), a sister species to *B. tryoni*, was not considered to be a pest (Drew, 1989), having been reared from just four commercial crops: peach,

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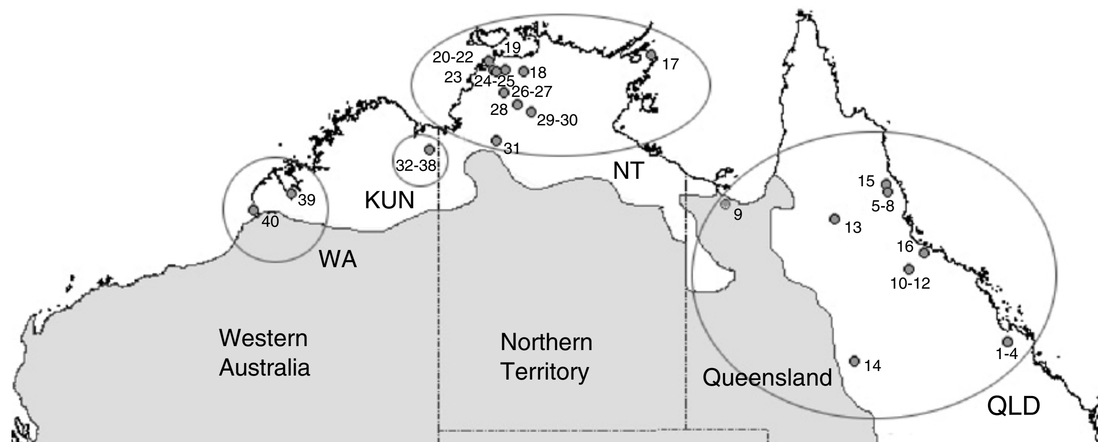

Fig. 1. Map of Australia showing the sampling sites in Queensland, Western Australia and the Northern Territory. Numbers correspond to locations given in table 1. Circles indicate the regions used in the analysis. The arid region separating Queensland and the Northern Territory is shaded.

guava and two species of citrus (Allwood & Angeles, 1979). However, in 1985, a large increase in the range of preferred hosts was reported for *B. aquilonis* in the Darwin area (Smith *et al*., 1988). Collections of fruit before and after this date showed that 40 cultivated fruit species, including mangoes and star fruit, were now being infested. Pest flies were soon found in other major towns in the Northern Territory and, in 2000, new outbreaks of pest flies were reported in the horticultural regions of Kununurra (834 km southwest of Darwin: S. Smith, personal communication).

The cause of the increase in infestation of commercial crops since 1985 is unknown and is complicated by the fact that *B. tryoni* and *B. aquilonis* are morphologically very similar. The first specimens of *B. aquilonis*, collected in Darwin in 1961, were identified as *B. tryoni* (May, 1963), before being described as a separate species based on their paler colouration and small differences in markings on the frons, wings, thorax and abdomen (May, 1965). The characters used to differentiate the species have since been questioned as being inconsistent and within the range of natural variation seen in *B. tryoni*. Nevertheless, the species status of *B. aquilonis* was maintained after a study by Drew & Lambert (1986). They found minor differences in the ovipositor, male genitalia and egg chorion. However, these differences are not easily observed, and classification is generally based on trapping location. *B. tryoni* and *B. aquilonis* produce viable and fertile hybrids in the laboratory (Drew & Lambert, 1986).

The morphological similarity between the two species has led to speculation that the recent outbreaks of pest flies in northwestern Australia may be the result of an invasion of *B. tryoni* or that a distinct new strain of *B. aquilonis* has adapted to utilize commercial crops as hosts (Wang *et al*., 2003; Raphael *et al*., 2004). A third possibility is that a hybridisation event between invading *B. tryoni* and *B. aquilonis* has occurred (Osborne *et al*., 1997; Morrow *et al*., 2000; Yu *et al*., 2001).

Previous studies have been inconclusive in identifying the cause of outbreaks in the northwest. An Australia-wide trapping survey conducted in early 1994 failed to find any flies unambiguously identified by morphology as *B. aquilonis*, suggesting that *B. tryoni* may have invaded the northwest (Osborne *et al*., 1997). However, studies using microsatellites have shown significant differences between east coast and northwest flies. Yu *et al*. (2001) studied flies collected over five years in Darwin and Queensland and showed significant differentiation at the six microsatellite loci analysed. Wang *et al*. (2003), using 30 microsatellite markers, found that east coast *B. tryoni* and samples from Darwin and Kununurra were all equally genetically differentiated from each other (F_{ST} = 0.01–0.02). That degree of differentiation was smaller than that measured between east coast samples of *B. tryoni* and another sibling species, *B. neohumeralis* ($F_{ST} \sim 0.08$). In contrast, mitochondrial DNA sequencing revealed sequence differences between east coast flies and a sample from Kununurra in the northwest, but not between *B. tryoni* and *B. neohumeralis* (Morrow *et al*., 2000). The aim of the present study was to determine whether a comprehensive DNA microsatellite survey could differentiate populations of *Bactrocera* in the northwest region.

Materials and methods

Sampling

Four groups of flies were used in the analysis (fig. 1, table 1):

1. For use as reference samples, *B. tryoni* was collected in Queensland where it is endemic. Flies were collected between 1999 and 2003 from both the east coast and towns in the arid west near the border with the Northern Territory.
2. NT: These flies were collected across the Northern Territory by one of the authors (ECC) in March 2002 and October 2003, except for the Darwin 1999 and Gove (site 17) samples that were collected by the NT Department of Primary Industries, Fisheries and Mines. Traps were set up to catch flies in urban and natural areas along transects from Kakadu to Darwin (152 km), Darwin to Katherine (314 km) and Katherine to Kununurra (514 km). Traps were set in bush areas near creeks and rivers as flies are thought to follow water courses (Meats, 1981). Where possible, traps were set near Kakadu plum trees (*Terminalia ferdinandiana*), the favoured host of *B. aquilonis* (Smith *et al*., 1988).

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Table 1. Descriptive statistics for each sample of flies used in the study. Sample size averaged over loci (n), number of alleles averaged over loci (A), proportion of low frequency alleles over all loci (low freq alleles), number of alleles unique to that sample (unique alleles), reduction in allelic richness per locus (allelic richness) with significance given (*, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$; ref, reference sample), expected heterozygozity (He), observed heterozygozity (Ho) and the inbreeding coefficient (f). For each sample the data is combined over 14 loci except samples 1 and 32 (13 loci) and samples 39 and 40 (11 loci).

Sample	Location	Region	Year	n	A	Low freq alleles	Unique alleles	Allelic richness	H_e	H_o	f
1	Bris-Cairns	QLD	2000	81.77	10.85	0.74	19	Ref	0.69	0.64	0.07
2	Bris-Cairns	QLD	2001	53.14	8.29	0.61	6	−1.05	0.70	0.64	0.09
3	Bris-Cairns	QLD	2002	38.93	7.50	0.59	3	−1.18	0.67	0.58	0.13
4	Bris-Cairns	QLD	2003	38.00	7.93	0.63	15	−0.56	0.70	0.61	0.12
5	Atherton	QLD	1999	20.71	6.29	0.50	0	−0.45	0.72	0.68	0.06
6	Atherton	QLD	2001	12.50	5.50	0.45	1	−0.57	0.68	0.64	0.07
7	Atherton	QLD	2002	20.14	5.93	0.51	1	−1.03	0.67	0.62	0.07
8	Atherton	QLD	2003	13.29	6.36	0.56	0	0.06	0.69	0.64	0.06
9	Burketown	QLD	2003	6.43	4.36	0.30	0	−0.31	0.70	0.59	0.18
10	Charters Towers	QLD	1999	16.64	6.21	0.55	2	−0.04	0.70	0.68	0.02
11	Charters Towers	QLD	2000	22.71	6.50	0.51	0	−0.52	0.70	0.67	0.04
12	Charters Towers	QLD	2001	15.79	6.57	0.58	0	0.35	0.71	0.65	0.09
13	Georgetown	QLD	2003	10.50	5.07	0.41	0	−0.52	0.67	0.65	0.03
14	Longreach	QLD	2000	23.36	6.50	0.54	1	−0.64	0.70	0.66	0.06
15	Mareeba	QLD	2002	20.14	6.43	0.56	0	−0.35	0.68	0.62	0.09
16	Toonpan	QLD	2001	11.57	5.79	0.49	0	−0.13	0.69	0.64	0.08
17	Gove	NT	2002	15.21	5.29	0.45	2	−1.22	0.66	0.63	0.05
18	Kakadu	NT	2002	37.86	7.00	0.59	1	−1.25	0.66	0.62	0.06
19	Fogg Dam	NT	2002	19.29	6.43	0.50	1	−0.35	0.66	0.61	0.08
20	Darwin	NT	1999	19.57	6.07	0.47	0	−0.59	0.71	0.70	0.00
21	Darwin	NT	2002	36.00	7.29	0.57	1	−0.91	0.67	0.59	0.12
22	Darwin	NT	2003	18.93	5.57	0.40	0	−1.05	0.69	0.64	0.07
23	Berrimah	NT	2002	27.14	6.29	0.57	0	−1.26	0.66	0.59	0.10
24	Manton Dam	NT	2002	47.29	7.50	0.60	1	−1.37	0.66	0.62	0.06
25	Manton Dam	NT	2003	19.21	5.21	0.37	0	−1.48	0.65	0.62	0.05
26	Hayes Creek	NT	2002	25.07	6.14	0.55	2	−1.42	0.66	0.59	0.09
27	Hayes Creek	NT	2003	17.07	5.57	0.46	0	−0.92	0.65	0.59	0.09
28	Cullen River	NT	2003	19.43	6.00	0.51	0	−0.76	0.67	0.61	0.10
29	Katherine	NT	2002	26.79	6.00	0.49	0	**−1.73***	0.65	0.61	0.07
30	Katherine	NT	2003	19.64	5.79	0.37	0	−0.98	0.68	0.63	0.07
31	Timber Creek	NT	2003	32.86	6.43	0.57	3	**−1.63***	0.66	0.59	0.10
32	Kununurra	KUN	Late 2000	75.25	8.33	0.66	2	**−2.24***	0.65	0.56	0.13
33	Kununurra	KUN	Late 2001	45.64	7.36	0.60	0	**−1.58***	0.68	0.61	0.10
34	Kununurra	KUN	Early 2002	18.07	5.93	0.54	0	−0.83	0.64	0.58	0.10
35	Kununurra	KUN	Mid 2002	24.50	5.50	0.49	0	−1.56	0.61	0.50	0.17
36	Kununurra	KUN	Late 2002	40.36	6.64	0.57	0	**−1.79***	0.63	0.54	0.14
37	Kununurra	KUN	Early 2003	33.79	6.00	0.51	1	**−2.00****	0.65	0.57	0.13
38	Kununurra	KUN	Late 2003	45.64	7.29	0.58	0	−1.52	0.65	0.55	0.15
39	Derby	WA	2001	16.73	4.45	0.37	0	**−3.26****	0.62	0.58	0.06
40	Broome	WA	2001	20.45	5.18	0.51	0	**−3.40***	0.60	0.53	0.12

3. KUN: The third group consisted of outbreaks of flies trapped in Kununurra, a developing horticultural region. Since 2000, large numbers of *B. aquilonis* have been trapped on an extensive trapping grid maintained by the Department of Agriculture and Food, Western Australia.
4. WA: Flies trapped by the WA department of Agriculture and Food in 2001 from Broome and Derby, isolated towns on the coast of Western Australia. *B. tryoni* had previously been eradicated from WA (in 1990) and these two populations are presumed to have been recently founded.

Standard cue-lure traps were used to collect the samples (White & Elson-Harris, 1992; Osborne *et al.*, 1997). Since females are not attracted to cue lure, all samples consisted of males only. The Queensland samples (QLD, samples 1–16) were each from single traps, except for samples 1–4 which were combined from numerous traps along the east coast, between Brisbane and Cairns. Yu *et al.* (2001) and Wang *et al.* (2003) have previously shown that there is no detectable population structuring along the entire Queensland coast.

The 15 Northern Territory samples consisted of flies caught in up to four traps within 70 km of each other in a ten-day period (table 1) with no more than 20 flies from any single trap being analysed. The one exception was the Timber Creek sample, which was a combination of 18 traps set up between Katherine and Kununurra (390 km). The samples from Kununurra (samples 32–38) were combined over four-month periods from traps placed throughout the town. The validity of grouping traps into samples was checked using Hardy-Weinberg tests.

Microsatellites

Total DNA was extracted from each fly using the Chelex method (Walsh *et al.*, 1991), and was typed using standard

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fluorescent PCR methods as described in Yu *et al*. (2001) at 14 polymorphic loci: Bt10, Bt11, Bt14, Bt15, Bt17, Bt32 (Kinnear *et al*., 1998, renamed in Yu *et al*., 2001), Bt1.1a, Bt2.6a, Bt2.6b, Bt6.12a, Bt4.1a, Bt8.12a, Bt8.6a (Wang *et al*., 2003), Bp78 (Shearman *et al*., 2006). Four of the samples had been used in a previous study and had been scored at fewer loci than were used in the current study; sample 1, 13 loci (no Bp78); sample 32, 13 loci (no Bt8.12a); and samples 39 and 40, 11 loci (no Bt15, Bt17 or Bt2.6b).

Each locus and sample was tested for departure from Hardy-Weinberg equilibrium and linkage disequilibrium in Genpop 3.3 (Raymond & Rousset, 1995), correcting for multiple comparisons using the sequential Bonferroni test.

Summary statistics for each sample were calculated using the GDA software (Lewis & Zaykin, 2002). The number of alleles, proportion of low frequency alleles (those with an allele frequency less than 0.1) and number of unique alleles were counted. Allelic richness was estimated taking the Qld 2000 sample (sample 1) as the reference population. Expected allele numbers for the given sample sizes were calculated by rarefaction (Hurlbert, 1971; Leberg, 2002). The significance of any changes from the expected values was tested using a resampling technique; 5000 resamples of the test sample size were drawn from the reference population, and the number with fewer alleles than the test sample was counted. Results were combined over loci (Sokal & Rohlf, 1995) and are presented as the average reduction in allele number per locus. These calculations were performed in Excel Visual Basic.

Differentiation between samples

F_{ST} values were calculated between each sample pair, and their significance was tested using the exact test procedure in FSTAT 2.9.3 (Goudet, 2002) with 5000 permutations. Population differentiation was also tested using chi-squared tests summed over loci (Yu *et al*., 2001). A hierarchical analysis of molecular variance (AMOVA) was performed in ARLEQUIN 2.0 (Schneider *et al*., 2000) to determine how much of the variation present in the complete data-set was due to between-region variation compared to within-region and within-sample variation. To calculate the significance of the variance components, 5000 resamples were used.

Spatial structuring was investigated by comparing genetic distance with geographic distance using the isolation by distance procedure implemented in GENEPOP ON THE WEB (Raymond & Rousset, 1995; Rousset, 2000) with 5000 permutations. In order to remove sample pairs with a geographic distance of 0, only one sample from each location was used. Samples from 2002 or, if 2002 was not available, 2001 were included. To visualize the genetic structuring, correspondence analysis using all samples was conducted in JMP (SAS Institute, 1994).

To further investigate population structure, the Bayesian clustering program STRUCTURE 2.1 (Pritchard *et al*., 2000; Falush *et al*., 2003) was used. STRUCTURE assigns individuals to clusters which are in Hardy-Weinberg and linkage equilibrium based on a user-defined number of clusters (K). Highly repeatable results were obtained with 50,000 iterations after a burn-in of 50,000 iterations and without prior population information.

Estimates of the rate of migration between the QLD and NT were calculated using the program BAYESASS 2.1 (Wilson & Rannala, 2003), which uses a Bayesian multilocus method to estimate the recent rate of migration expressed as a proportion of the recipient population.

Mitochondrial sequence analysis

Mitochondrial sequences from the *cytochrome b* (*cytb*) (Kocher *et al*., 1989) and *cytochrome oxidase II* (*COII*) (Simon *et al*., 1994) genes were amplified in individual flies from the northwest and Queensland as described in Morrow *et al*. (2000). PCR products were purified using the Wizard® SV gel and PCR clean-up system (Promega, USA) before being sequenced. Sequences were aligned using SEQUENCHER™ 4.1 (Gene Codes) and the number of mitotypes (mitochondrial haplotypes) scored.

Five flies from Darwin 2002, Katherine 2002 and Kununurra 2000 and one fly from Kakadu 2002 and Kununurra 2001 were compared to a sample of five flies from west Queensland. Comparisons were made between these sequences and those from the previous study by Morrow *et al*. (2000).

Results

Summary statistics

A total of 1159 flies from 40 samples were scored at 11 to 14 microsatellite loci (table 1). The number of alleles at each locus ranged from 5 to 41, with heterozygozities between 0.30 and 0.88. After correcting for multiple comparisons (table size = 692, including all samples and all loci scored), two loci showed deviations from Hardy-Weinberg equilibrium in a small number of samples. Bt15 was not in equilibrium in seven samples (samples 1, 4, 12, 14, 32, 36 and 38) and Bt32 in five samples (samples 4, 18, 21, 32 and 36). In a previous study by Yu *et al*. (2001), Bt15 was shown to have a low frequency of null alleles in Queensland populations. Consequently, Bt15 and Bt32 were excluded from those analyses which assume equilibrium. One other locus, 2.6b, showed an excess of homozygotes in sample 24. Two pairs of loci showed significant linkage disequilibrium in 1–2 samples; loci 2.6b and 2.6a in samples 32 and 35 and 2.6b and 8.6a in sample 32. These samples are both outbreak samples from Kununurra. Excluding these loci did not change the results presented; thus, they were included in the analyses.

Descriptive statistics were uniform across the range of samples (table 1). The number of alleles and unique alleles were, as expected, greater in the large samples from Queensland. Allelic richness decreased most in samples from Kununurra and WA, with the most westerly samples being the most depleted. Several of the northwest samples had alleles not found in Queensland.

Differentiation between samples

Within the Northern Territory samples, genetic differentiation was minimal with only one of 105 pairwise F_{ST} estimates significant ($P < 0.001$). Those two samples were separated by four years and 350 km (samples 20 and 31; table 2). Similar spatial and temporal homogeneity was shown among the Queensland samples. Differentiation between the Northern Territory and Queensland regions was greater, with the overall $F_{ST} = 0.015$, $P < 0.05$; however, most pairwise comparisons were non-significant.

In contrast, the Kununurra samples showed significant heterogeneity across years (table 2) with corresponding

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Table 2. Results of genetic differentiation tests between all pairs of fly samples. The significance of chi-squared tests of heterogeneity is given below the diagonal by asterisks (NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Values above the diagonal are FST estimates. Significant values are in bold and underlined. All tests were corrected for multiple comparisons. Sample numbers and names correspond to those in table 1. Boxed results include samples from the same region.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	QLD		0.003	0.003	0.005	-0.002	0.002	0.003	0.003	0.023	0.002	0.007	0.004	-0.007	-0.005	0.000	-0.001	0.008	0.008	0.004	0.017	0.008	0.010	0.011	**0.020**	**0.012**	0.013	0.013	0.006	**0.005**	0.006	**0.015**	**0.022**	**0.018**	0.009	**0.039**	**0.017**	**0.027**	**0.011**	**0.093**	**0.022**
2	QLD	NS		0.000	0.003	0.002	0.002	0.002	0.007	0.000	-0.001	0.009	-0.001	0.001	0.004	-0.001	0.003	0.003	**0.011**	0.007	0.013	0.014	0.005	0.019	**0.012**	**0.022**	0.015	0.026	0.008	0.007	0.012	0.021	**0.026**	**0.013**	0.024	**0.035**	**0.027**	0.027	**0.019**	**0.108**	**0.035**
3	QLD	NS	NS		0.008	0.005	0.004	0.001	0.006	-0.002	0.006	0.006	0.008	0.004	0.004	-0.004	0.003	0.003	**0.008**	0.001	0.016	0.016	0.004	0.016	**0.011**	0.015	0.013	0.024	0.006	0.011	0.010	0.011	**0.021**	**0.013**	0.018	**0.031**	**0.022**	0.021	0.011	**0.099**	**0.030**
4	QLD	NS	NS	NS		0.007	0.005	0.017	0.022	-0.001	0.006	0.012	0.013	0.011	0.005	0.002	0.002	0.008	**0.016**	0.017	0.018	0.022	0.014	0.026	**0.021**	**0.024**	0.013	0.028	0.020	**0.017**	0.019	**0.023**	**0.024**	**0.022**	0.022	**0.044**	**0.030**	**0.030**	**0.025**	**0.093**	**0.032**
5	QLD	NS	NS	NS	NS		0.008	0.007	0.012	0.004	0.001	0.005	0.003	0.004	-0.001	0.005	0.017	0.009	**0.014**	0.016	0.007	0.017	0.005	0.019	**0.019**	0.020	0.020	0.021	0.009	**0.014**	0.007	**0.022**	0.025	0.017	0.014	**0.045**	**0.023**	0.029	**0.018**	**0.110**	**0.039**
6	QLD	NS	NS	NS	NS	NS		0.007	0.016	0.006	0.014	0.013	0.008	0.011	0.001	0.001	-0.001	0.007	0.013	0.006	0.016	0.015	0.011	0.032	0.021	0.020	0.008	0.016	0.015	0.007	0.007	0.016	**0.033**	0.021	0.018	0.051	0.026	0.025	0.014	0.096	**0.033**
7	QLD	NS	NS	NS	NS	NS	NS		0.001	0.007	0.011	0.012	0.004	0.001	0.010	0.000	0.010	0.005	0.007	0.006	0.015	0.013	0.004	0.014	0.008	**0.021**	0.012	0.015	0.006	0.007	0.012	**0.020**	**0.021**	0.011	0.020	**0.029**	**0.021**	0.017	0.015	0.128	**0.020**
8	QLD	NS	NS	NS	NS	NS	NS	NS		-0.017	0.012	0.009	-0.013	0.000	0.003	0.004	0.001	0.024	0.023	0.016	0.025	0.027	0.022	0.029	0.018	0.029	0.027	0.024	0.010	0.019	0.023	0.031	0.008	0.018	0.040	0.045	0.036	0.022	0.023	0.105	0.035
9	QLD	NS	NS	NS	NS	NS	NS	NS	NS		0.003	0.013	-0.011	0.009	-0.005	-0.005	0.003	0.024	0.017	0.022	0.020	0.036	0.011	0.028	0.017	0.024	0.022	0.020	0.006	0.028	0.019	0.033	0.020	0.015	0.032	0.040	0.029	0.017	0.021	0.115	**0.086**
10	QLD	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.001	0.004	-0.001	-0.001	-0.005	0.006	0.021	**0.022**	0.019	0.019	0.027	0.010	0.035	**0.028**	**0.039**	0.025	0.048	0.015	**0.025**	0.026	**0.028**	**0.042**	0.028	0.033	**0.052**	0.034	0.047	**0.031**	**0.117**	**0.044**
11	QLD	NS	*	NS	NS	NS	NS	NS	NS	NS	NS		0.005	0.005	0.004	-0.005	0.005	0.017	**0.020**	0.010	**0.024**	0.027	0.012	0.026	**0.024**	**0.028**	0.025	0.033	0.019	**0.025**	0.021	0.016	**0.037**	0.020	0.032	**0.044**	**0.033**	0.030	**0.026**	**0.094**	**0.034**
12	QLD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.002	0.001	0.004	0.004	0.013	0.013	0.011	0.016	0.012	0.010	0.019	**0.014**	0.017	0.015	0.015	-0.001	0.010	0.014	0.023	0.018	0.010	0.022	0.033	0.021	0.015	0.017	**0.107**	**0.045**
13	QLD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.007	0.004	0.010	0.016	0.012	0.013	0.006	0.012	0.009	0.021	0.015	0.026	0.016	0.030	-0.004	0.009	0.012	0.013	0.029	0.022	0.027	0.059	0.039	0.021	0.018	0.115	0.017
14	QLD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.001	0.005	0.009	**0.013**	0.011	0.011	**0.017**	0.006	**0.030**	**0.021**	**0.017**	**0.019**	**0.024**	0.011	**0.014**	0.011	**0.017**	**0.015**	**0.023**	0.018	0.049	**0.028**	0.024	**0.016**	**0.088**	**0.018**
15	QLD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.002	-0.002	0.007	0.000	0.013	0.014	-0.001	0.019	0.007	0.015	0.005	0.022	0.006	0.006	0.004	0.006	0.013	0.008	0.015	0.022	0.013	0.018	0.012	0.100	0.021
16	QLD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.015	0.023	0.005	0.020	0.024	0.018	0.037	0.028	0.036	0.019	0.035	0.024	0.022	0.025	0.026	**0.026**	0.021	0.038	0.052	0.041	0.035	0.029	0.079	0.027
17	NT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.011	-0.003	0.007	-0.009	-0.007	0.003	-0.008	-0.010	-0.005	0.007	0.002	-0.010	-0.011	0.001	0.006	-0.001	-0.002	0.022	0.011	-0.001	-0.008	0.108	0.015
18	NT	NS	***	*	***	**	*	*	***	**	***	***	**	*	**	NS	***	NS		-0.003	0.008	-0.003	-0.001	-0.001	-0.004	-0.004	-0.003	0.006	-0.001	-0.003	-0.004	0.003	0.006	0.000	0.000	**0.025**	0.011	-0.001	-0.003	**0.111**	0.016
19	NT	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.018	0.002	0.002	0.007	0.006	0.006	0.000	0.013	0.004	0.005	0.000	0.000	0.011	0.002	0.009	0.025	0.019	0.011	-0.002	0.082	0.014
20	NT	**	**	NS	*	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS		0.007	-0.001	0.016	0.013	0.023	0.012	0.028	0.005	0.006	0.006	0.019	0.022	0.014	0.017	**0.051**	0.030	0.017	0.016	**0.113**	**0.042**
21	NT	***	**	***	***	**	*	NS	***	**	***	***	NS	NS	***	NS	***	NS	NS	NS	NS		0.003	-0.005	0.000	-0.003	-0.001	0.010	0.004	-0.004	-0.005	0.006	0.011	0.004	0.003	0.031	0.018	0.005	0.000	**0.116**	0.012
22	NT	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.010	0.002	0.008	0.007	0.019	0.006	0.001	0.003	0.012	0.017	0.006	0.007	0.023	0.011	0.012	0.004	**0.098**	0.034
23	NT	NS	**	NS	***	NS	**	NS	**	NS	***	*	*	NS	***	NS	***	NS	NS	NS	NS	NS	NS		-0.003	0.002	0.002	0.008	0.003	0.009	0.001	0.007	0.008	-0.001	0.012	0.033	0.021	0.005	0.005	**0.116**	0.014
24	NT	***	***	**	***	***	*	NS	**	**	***	***	***	NS	***	NS	***	NS	NS	NS	NS	NS	NS	NS		0.000	0.000	0.009	0.000	0.000	0.000	0.008	0.008	-0.001	0.011	**0.023**	**0.017**	0.000	0.002	**0.127**	0.031
25	NT	**	**	NS	***	NS	NS	*	NS	NS	***	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS		0.001	0.001	0.003	-0.001	-0.010	0.002	0.000	0.004	-0.008	0.022	0.008	-0.004	-0.011	0.098	0.008
26	NT	**	**	**	**	*	NS	NS	**	NS	**	**	NS	NS	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.008	0.001	-0.002	-0.007	-0.002	0.014	0.002	0.002	**0.034**	0.016	0.003	-0.002	**0.085**	0.016
27	NT	NS	***	*	***	NS	NS	NS	NS	NS	***	**	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.006	0.005	0.006	0.011	0.008	0.009	0.010	0.034	0.012	0.004	0.002	0.109	0.023
28	NT	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.001	-0.004	0.001	0.017	0.001	0.012	0.041	0.018	0.011	0.001	**0.110**	0.025
29	NT	NS	NS	NS	**	*	NS	NS	*	***	***	***	*	NS	***	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.005	0.006	0.012	0.007	-0.003	**0.027**	0.012	0.007	-0.002	**0.102**	0.014
30	NT	NS	NS	NS	*	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		-0.005	0.004	0.000	-0.004	**0.036**	0.017	-0.002	-0.007	**0.088**	0.007
31	NT	***	***	**	***	***	*	***	***	***	***	**	***	NS	***	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.026	0.008	0.011	**0.042**	0.023	0.009	0.000	**0.088**	0.011
32	KUN	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***	NS	NS	NS	***	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	**		0.009	0.005	**0.018**	**0.015**	0.001	0.008	**0.119**	**0.040**
33	KUN	***	***	***	***	***	**	**	***	**	***	***	**	***	***	NS	***	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.008	0.017	0.011	0.005	0.005	**0.115**	**0.026**
34	KUN	NS	*	NS	**	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.018	-0.002	0.012	-0.005	0.098	0.013
35	KUN	***	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***	**	***	***	***	***	***	***	***	**	***	***	***	***	***	***	***	***	**		0.000	0.026	**0.014**	**0.126**	**0.031**
36	KUN	***	***	***	***	***	***	***	***	*	***	***	***	***	***	*	***	NS	*	*	***	***	NS	***	***	NS	**	NS	NS	***	*	***	***	NS	NS	NS		0.033	**0.030**	**0.156**	**0.053**
37	KUN	***	***	*	***	***	**	NS	**	*	***	***	**	*	***	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	***		0.003	0.118	0.034
38	KUN	***	***	**	***	***	NS	**	***	NS	***	***	***	**	***	*	***	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	***	NS		0.085	0.017
39	WA	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***		**0.104**
40	WA	NS	***	*	***	**	*	NS	**	***	***	***	***	NS	*	NS	**	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	***	**	NS	NS	***	

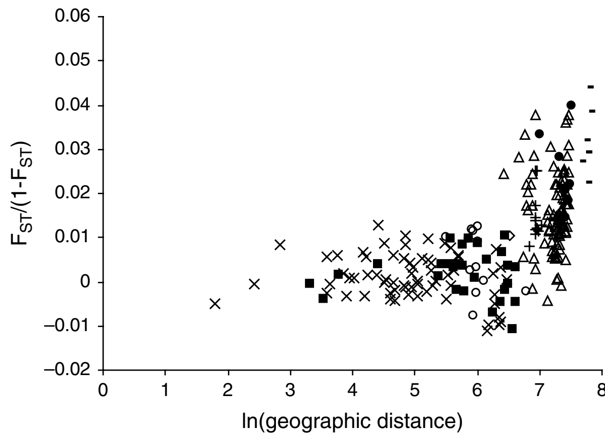

Fig. 2. Geographic distance (as ln(geographic distance)) plotted against genetic distance (as $F_{ST}/(1–F_{ST})$) calculated between samples of flies. Only one sample from each location was included to eliminate comparisons with a geographic distance = 0. QLD refers to comparisons made between samples within this region, similarly for NT. All other comparisons were made between regions (■, QLD; X, NT; △, NT-QLD; ●, KUN-QLD; ○, KUN-NT; –, QLD-Broome; +, NT-Broome; ◇, KUN-Broome).

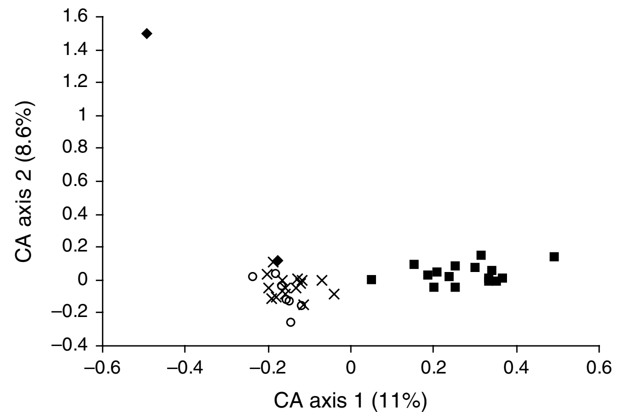

Fig. 3. Correspondence analysis of all fly samples plotted on the first and second axes (% of variation explained by each axis). Samples from the different regions are indicated by different shapes (○, KUN; X, NT; ■, QLD; ◆, WA). Of the two WA samples (Derby and Broome), the Derby sample (sample 39) was separated from all others.

variation in genetic distance to the Queensland and Northern Territory samples. Despite being only 165 km apart, the two WA samples were highly differentiated: $F_{ST} = 0.10$, $P < 0.001$. Sample 39 (Derby) was significantly differentiated from all other samples, while sample 40 (Broome) was similar to the Northern Territory samples.

A Mantel test conducted by comparing geographic distance with $F_{ST}/(1–F_{ST})$ was highly significant ($P < 0.001$; fig. 2). Sample 39 (Derby) was not included in this analysis since it showed strong founder effects. When the Queensland samples were excluded, this regression was no longer significant ($P = 0.6$).

A correspondence analysis separated the samples into two main groupings (fig. 3). One consisted of the Queensland samples and the other of the Northern Territory, Kununurra and Broome samples. The groupings were separated by the first axis, which explained 11% of the variation in all the data. The Derby sample was separated from all other samples on both the first and second axes (8.6% of variation). AMOVA showed that 99.2% of the variation was within the samples ($P < 0.001$) with no significant variation either between regions (i.e. Queensland versus northwest samples, excluding Derby) or between samples within regions. The rate of migration, m, estimated to be occurring from Queensland into the Northern Territory was 0.012 (standard deviation = 0.008).

Clustering analysis, using the program STRUCTURE, included all samples. Although the number of clusters (K) was varied from K = 1 to 8, the posterior probabilities did not sharply plateau (fig. 4a). At K = 2, the Queensland samples were incompletely separated from the Northern Territory samples (fig. 4b). Of the Queensland individuals, 59% were assigned to cluster 1 with probability greater than 0.9. At this level, 4% of the Queensland samples were assigned to cluster 2 while 37% were not assigned to any cluster. Of the northwest samples, 38% were assigned to cluster 2 while 6% were assigned to cluster 1 and 56% remained unassigned. Reanalysis with the Queensland samples removed revealed no further groupings among the northwest samples, although at K = 4 a possible cluster was found that included some of the Kununurra samples.

mtDNA sequencing

Mitochodrial DNA sequences from the *cytb* region of 22 individuals (GenBank accession numbers GQ121835–GQ121856) were combined with the sequence data from Morrow *et al*. (2000) to give a total of 41 samples from Queensland and 26 from the northwest (including Kununurra) (fig. 5). Over the 240 bp sequenced, 28 mitotypes were observed. The most common mitotype found throughout the northwest (5/26) was also found in Queensland (4/41). Only one other mitotype, the consensus sequence, was shared between the regions. Sixteen samples had a unique mitotype. Previous analysis of flies from Kununurra had found that site 166 was fixed for G (Morrow *et al*., 2000). In the current study, again, all flies from Kununurra have this G, as do some but not all the NT samples (7/11).

From the *COII* region, 500 bp were sequenced in 16 individuals from the northwest and four from Queensland (Genbank accession numbers GQ121857–GQ121876); 176 bp overlapped with the sequences of Morrow *et al*. (2000). A total of 22 mitotypes were present in the combined dataset (fig. 6) with two mitotypes, including the consensus sequence, shared between the two regions. Base 246, which was fixed for G in the three Kununurra flies sequenced by Morrow *et al*. (2000) was either a G or an A in the individuals sampled here and was present in one of the Queensland samples. The results here show no obvious separation between the Queensland and northwest samples.

Discussion

Genetic differentiation

The genetic distance between the Northern Territory and Queensland samples was small ($F_{ST} = 0.015$), consistent with

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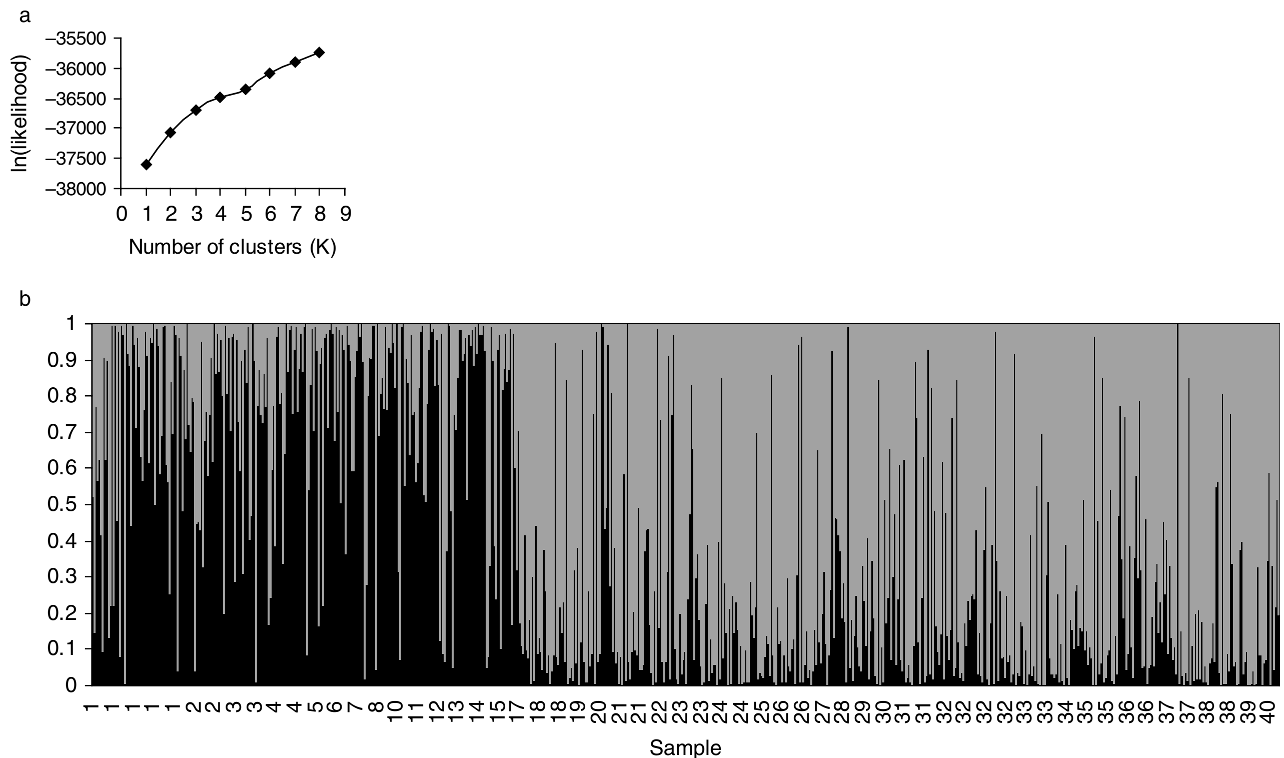

Fig. 4. Results of Bayesian clustering analysis between all QLD (1–16), NT (17–31), KUN (32–38) and WA (39–40) samples: (a) posterior probabilities of K, the number of clusters, and (b) the probability at K = 2 with which each individual is assigned to each of the two clusters. The sample numbers refer to those in table 1. For larger samples, the sample number is listed repeatedly to indicate which individuals belong to which sample.

low levels of migration from Queensland into the Northern Territory. This degree of differentiation is within the range of genetic distances observed in eastern Australia between various populations of *B. tryoni* (F_{ST} = 0.002–0.08: Sved *et al*., 2003, Wang *et al*., 2003). This finding suggests that the two Northern Territory and Queensland samples have only recently separated and/or that there has been continuing gene flow since separation.

Within the Northern Territory, a high level of homogeneity was found despite the inclusion of flies from urban, horticultural and natural areas. Samples were not only homogeneous over time but also showed no evidence of spatial structuring. The only significant difference between localities occurred between the Darwin sample from 1999 and the combined sample from traps around Timber Creek in 2003. This may have been due to the relatively low population densities around Timber Creek. Similarly, no structuring among samples was evident using the Bayesian clustering analysis or the correspondence analysis.

There was no evidence of isolation by distance in the Northern Territory. These findings are contrary to the proposition that *B. tryoni* is present in Darwin but *B. aquilonis* is present outside that urban area (Smith *et al*., 1988; Osborne *et al*., 1997; Morrow *et al*., 2000; Wang *et al*., 2003). These results suggest that just one strain is present in the Northern Territory. A similarly extensive and stable population has already been shown to exist in Queensland (Yu *et al*., 2001).

The samples of flies from Kununurra varied between years and showed differing levels of differentiation from the Northern Territory samples. In clustering analyses, they grouped mainly with the Northern Territory samples, suggesting they originate from the Northern Territory. Trapping in the drier areas between the Northern Territory and Kununurra regions yielded very few or no flies. Thus, it is likely that the Kununurra population is isolated but with occasional migration from the Northern Territory region. For the WA samples, those from Derby showed evidence of founder effects in contrast to the Broome sample, which grouped closely with the Northern Territory flies. A similar fragmentation of *B. tryoni* populations was found in the drier areas of eastern Australia, where *B. tryoni* is restricted to urban areas in which artificial irrigation provides necessary moisture (Dominiak *et al*., 2006). These populations are also often short lived and subject to strong founder effects (Meats *et al*., 2003; Sved *et al*., 2003).

Mitochondrial DNA

Morrow *et al*. (2000) carried out an analysis of two mitochondrial sequences (*cytb* and *COII*) in *B. tryoni* and a limited number of flies from Kununurra classified as *B. aquilonis*. They found evidence of fixed differences between the two species. However, using a wider sampling regime, our sequence data provided no evidence for the separation of northwest samples from the Queensland

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```
                 1111111222222222
                8801256992333458899
                2395416399258133656

                ATACATATACTATAAAACC

Dwn-1           G.....G.......G....
Dwn-2           ......G.......G....
Dwn-3           ......G......GG....
Dwn-4           G.....G......GG....
Dwn-5           ...................
Kak             ......G.......G....
Kth-1           ..............G....
Kth-2           ........G.....G....
Kth-3           ..............G....
Kth-4           ......G.......G.G..
Kth-5           ......G.......G....
Kun00-1         G.....G.......G....
Kun00-2         G.....G.......G....
Kun00-3         ......G............
Kun00-4         ......G.......G....
Kun00-5         ......G............
Kun01           ......G......GG....
*KUN- D5.2      ......G......G.....
*KUN- D5.3      ......G.......G....
*KUN- D5.4      ......G......G.....
*KUN- D5.5      ......G......G.....
*KUN- D5.6      ......G.....C.G....
*KUN-A325       ......GC......G..T.
*KUN-A363       ......G.......G...T
*KUN-A395       ......G......G.....
*KUN-D5.1       ......G.......G....
QlD-1           ................G..
QlD-2           ................G..
QlD-3           G..........G.......
QlD-4           ................G..
QlD-5           ................G..
*QLD-446.1d     ...................
*QLD-481.1      ....T..............
*QLD-481.2      ......G.........G..
*QLD-481.3      ...T...............
*QLD-TC1        ...................
*QLD-TC2        ...................
*QLD-TC3        ......G.......G....
*QLD-300.1      ...................
*QLD-300.2      .....G..........G..
*QLD-300.3      ...................
*QLD-311.1      .........T.....G...
*QLD-311.2      ................G..
*QLD-311.3      .........T.....GG..
*QLD-TT1        ................G..
*QLD-TT2        ...................
*QLD-TT3        ...........G.......
*QLD-TT4        ..T.............G..
*QLD-TN1        ................G..
*QLD-TN2        ................G..
*QLD-TN3        .C............G....
*QLD-TN4        ...................
*QLD-49.1       .........T.....G...
*QLD-49.2       ................G..
*QLD-49.3       GC.......TC....G...
*QLD-47.1       ......G.......G....
*QLD-47.2       ...................
*QLD-47.3       .........T.....G...
*QLD-TB2        G........T.....G...
*QLD-TB4        ......G.......G....
*QLD-TB5        .........T.....GG..
*QLD-448.1c     ................G..
*QLD-448.2c     ...................
*QLD-448.3c     ......G.......G....
*QLD-449.1      ...........G.......
*QLD-449.2      ................G..
*QLD-449.4      ......G..T.....GG..
```

Fig. 5. Polymorphic sites in the sequenced segment of the *cytb* gene. Samples are from Darwin (Dwn), Kakadu (Kak), Katherine (Kth), Kununurra (Kun00, Kun01) and Queensland (QLD). Samples marked with a * are from Morrow *et al.* (2000). Nucleotides are numbered from the same starting point as Morrow *et al.* (2000). The figure shows the polymorphic sites, their position and the common and alternative base at each site.

```
             2 2 2 2 2 2 2 3 3 4 4 4 4 4 5 5 6
             1 1 4 5 7 8 8 1 3 0 1 3 6 6 0 7 1
             6 9 6 4 3 3 5 2 0 5 7 8 5 8 1 3 2

             A A A T A T C G A T A A G A T G T

Dwn-1        . . . . . . . . . . . G . . . . .
Dwn-2        . . . . . . . . . . . . . . . . .
Dwn-3        . . . . . . . . G . . . . . . . .
Dwn-4        . . . . . . . . . . . . . . . . .
Dwn-5        . . . . . . . . . . . . . . . . .
Kak          . . . . . . . . . . G . . . . . .
Kth-1        . . . . . . . . . . . . . . . . C
Kth-2        . . . . . . . . . . . . . . . A .
Kth-3        . . . . . . . . . . . . . . C . .
Kth-4        G . . . G G T . . . . . . . . . .
Kun00-1      . . . . . . . . . . . G . . . . .
Kun00-2      . . G . . . . . . . . . A T . . .
Kun00-3      . . . . . . . . . C . . . . . . .
Kun00-4      . . . . . . . . . . . . A T . . .
Kun00-5      . T . . . . . . . . . . . . . . .
Kun01        . . . . . . . . G . . . . . . . .
*Kun-A325    . . G . . . . .
*Kun-A363    . . G . . . . .
*Kun-D5.1    . . G . . . . .
Qld-1        . . G . . . . . . . . . A T . . .
Qld-3        . . . . . . . . . . . . . . . . .
Qld-4        G . . . . . T . . . . . . . . . .
Qld-5        G . . . . . T A . . . . . . . . .
*Qld-TC1     . . . . G . . .
*Qld-TC2     . . . . G . . .
*Qld-TC3     . A . C G . . .
*Qld-TC4     G . . . . T . A
*Qld-TC5     . . . . . . . .
*Qld-TC6     . . . . . . . .
*Qld-TT1     G . . . . . . A
*Qld-TT3     . . . . G . . .
*Qld-TN1     G . . . . . . .
*Qld-TN4     . . . . G . . .
*Qld-TN5     . . . . . . . .
*Qld-TB2     . . . . . . . A
*Qld-TB4     . . . . . . . .
*Qld-TB5     . . . . . . . A
*Qld-TB6     . . . . . . . .
*Qld-TB7     . . . . G . . .
```

Fig. 6. Polymorphic sites in the sequenced segment of the *COII* gene. Samples are from Darwin (Dwn), Kakadu (Kak), Katherine (Kth), Kununurra (Kun00, Kun01) and Queensland (QLD). Samples marked with a * are from Morrow *et al.* (2000). Nucleotides are numbered from the same starting point as Morrow *et al.* (2000). The figure shows the polymorphic sites, their position and the common and alternative base at each site.

samples. The potential fixed difference between northwest and Queensland flies found by Morrow *et al.* (2000) was shown not to be fixed in the present study.

Species status of B. aquilonis

Despite the morphological differences found in previous studies (Drew & Lambert, 1986), the genetic data do not

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support the separation of the eastern and northwestern flies into two species. The level of genetic differentiation is similar to that observed between geographically close populations of other tephritid species (Mun *et al*., 2003; Bonizzoni *et al*., 2004; Augustinos *et al*., 2005; Nardi *et al*., 2005). Compared with previous studies in *B. tryoni*, the genetic distance found here between the Northern Territory and Queensland flies ($F_{ST} = 0.015$) is larger than that between endemic east coast populations of *B. tryoni* (average $F_{ST} = 0.002$) but smaller than that found between populations of *B. tryoni* in individual towns further south (average $F_{ST} = 0.03–0.08$: Sved *et al*., 2003). The genetic distance between *B. tryoni* and *B. neohumeralis* (another species pair that produce viable hybrids) is $F_{ST} = 0.085$ (Wang *et al*., 2003). Based on genetic distances alone, the Northern Territory and Queensland flies appear more likely to be allopatric populations of the same species.

It seems extremely unlikely that our sampling did not pick up flies that previously would have been classified as *B. aquilonis*. However, if we did not, then *B. aquilonis* must represent a very small proportion of the flies in this region. The sample from Darwin in 1999 used in this study had been morphologically identified by R. Drew (personal communication) as *B. aquilonis*. That group could not be distinguished from the other samples using molecular markers.

It is possible that *B. aquilonis* existed in the past and has now been replaced by *B. tryoni*. However, our sampling covered urban, horticultural and bush areas and no evidence of distinct populations was found in any habitat. If *B. tryoni* has displaced *B. aquilonis*, then replacement has occurred in all habitats in less than 30 years. This seems unlikely since trappings occurred in National Parks, large areas of intact natural habitat.

An alternative possibility is that *B. aquilonis* is not a separate species. Under this scenario, the flies first trapped in the Northern Territory in 1961 (May, 1963) would have been *B. tryoni*, and all morphological differences are within the natural range of variation of *B. tryoni*. One of the main differences used to differentiate *B. tryoni* and *B. aquilonis*, apart from distribution, is pest status, with *B. aquilonis* considered benign. However, we suggest that the change in pest status may be due to a large increase in population size following the expansion of the horticultural industry in the Northern Territory in the 1980s. Annual fruit production increased from 545 tonnes in 1981 to 1946 tonnes in 1983. This included an increase in mango production, a favoured host of *B. tryoni*, from an average of seven tonnes between 1977 and 1981 to 150 tonnes between 1983 and 1986, with most new plantings occurring in the Darwin region (Northern Territory Department of Primary Production, 1972–1991). This increase corresponds with the change in behaviour observed by Smith *et al*. (1988) of an increase in pest fruit fly activity in the Darwin region from 1985. The rise of the 'new' pest species in northwestern Australia may only reflect a large increase in population size resulting from increased host availability.

Wang *et al*. (2003) also discussed the question of whether *B. tryoni* and *B. aquilonis* were separate species. Their less comprehensive survey also found the two species to be minimally genetically differentiated. For the purposes of species definitions, they concluded that since the two groups are allopatric, it makes little difference whether they are considered one or two species. From a quarantine perspective, the genetic similarity between *B. tryoni* and the northwestern species would suggest that control and disinfestation protocols should be similar in both regions. Quarantine practices appropriate to *B. tryoni* outbreaks have been in place in the northwest region for a number of years (Yonow & Sutherst, 1998).

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