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Microsatellite analysis of the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae) indicates spatial structuring: implications for population control

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Abstract

The population structure of a tephritid pest species, the Queensland fruit fly *Bactrocera tryoni* (Froggatt), has been analysed over a five year period (1994–1998), using six microsatellites. Adult fly samples were collected to cover most regions of eastern and central Australia where the flies are regularly found. Tests for heterogeneity indicated that flies within geographically defined regions were homogeneous. The samples were allocated into five regions, including one very large region, Queensland, which encompasses that portion of the fly's range where breeding can occur year-round. With one exception, the collections from different regions were homogeneous between years, showing a fairly static distribution of the species. However, differences between regions were highly significant. The one case of a change in frequency between years indicated a gradual replacement of flies in a marginal region by flies from the main part of the range. The finding of stability in the distribution of a highly mobile insect is of interest, potentially also for other species which have expanded beyond their native range. It is argued that a contributing reason for this stability may be adaptation to different climatic regimes, and that strategies for control based on this hypothesis afford a reasonable chance of success.

Introduction

The major fruit fly pest in Australia is *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), the Queensland fruit fly. This species now has a permanent distribution along the entire east coast of Australia, and has the potential to infest virtually all fruit species grown commercially in eastern Australia (Drew, 1989).

It seems probable that the species was originally endemic to rainforests of the east coast of Australia, given that its native hosts include a large number of Queensland rainforest fruits (May, 1963). However, *B. tryoni* rapidly adapted to cultivated fruits, and was recorded as a pest species as early as 1897 (Froggatt, 1897). Today, the endemic range has clearly increased. *Bactrocera tryoni* occurs in a wide band over eastern Queensland and New South Wales, and extends into coastal Victoria (Meats, 1981). It is frequently recorded from inland areas that it has invaded only since the cultivation of introduced fruits (Fletcher, 1979). Furthermore, flies with the morphological and host-fruit characteristics of *B. tryoni* are found in the Northern Territory, at Darwin and Alice Springs (Osborne *et al.*, 1997).

Outbreaks have occurred in south-western New South Wales, inland Victoria, South Australia and Western Australia, and it is clear that *B. tryoni* has the potential to

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infest cultivated fruit in most of tropical and temperate Australia (Meats, 1989). Considerable effort is expended by the community and by agricultural and quarantine authorities to prevent expansion of *B. tryoni* into South Australia and the 'Fruit Fly Exclusion Zone' – an area which includes major fruit growing regions around the borders of New South Wales, Victoria and South Australia. Maintenance of 'fruit fly free' areas includes roadblocks, trapping grids to detect outbreaks and eradication of outbreaks detected. Despite these efforts outbreaks occur regularly in fruit fly free areas, such as Adelaide (Maelzer, 1990). Any outbreak results in loss of 'fruit fly free' status for horticultural products.

Bateman (1967) studied population structure of *B. tryoni* by looking for differentiation in heat and cold tolerance in flies sampled along the east coast of Australia. Although some flies, e.g. those collected from near Sydney, were shown to be slightly more tolerant to cold than those from further north, no straightforward cline was found and no simple conclusions about population structure could be drawn. McKechnie (1975) also studied the distribution using isoenzymes, but did not find evidence for population structuring.

As part of a new effort to investigate this problem, Kinnear *et al*. (1998) isolated a set of 16 microsatellites from *B. tryoni*. From this set, six microsatellites, with dinucleotide, trinucleotide or complex repeats, showed the highest levels of heterozygosity, suitable for population analysis. A preliminary report using these microsatellites (Yu *et al*., 2000), based on collections within the endemic range of the species in the years 1995 and 1996, concluded that the distribution of *B. tryoni* remained virtually unchanged for two years and that population differentiation could be detected, even within the continuous east coast distribution. The present analysis includes data for all the years 1994–1998, thus allowing a more accurate picture of the distribution to be drawn. The basic conclusion is of a differentiated population, with discrete and stable sub-populations, even in a continuous distribution of an extremely mobile pest species.

Materials and methods

Samples

Adult male flies were collected for the analysis using traps baited with a mixture of cue lure and malathion, as described in Osborne *et al*. (1997), which presents an account of the first collection made in 1994, involving over 1000 traps. Subsequent samples were obtained by the same methods, using a subset of the trapping sites. For the microsatellite analysis, a set of around 25 sites was chosen for each year. In most cases the same sites were used in all years, but in some cases it was necessary to substitute flies from nearby trapping sites. The diagram of trapping locations is shown in fig. 1. A maximum of 20 flies was analysed from any one trap.

Microsatellite screening

Six highly polymorphic microsatellite loci, previously isolated by Kinnear *et al*. (1998), were selected for the present study. Polymerase chain reaction (PCR) primers were designed to amplify a product 100–200 bp in length spanning each microsatellite locus. To simplify the notation, the loci have been re-designated as shown in table 1, which lists the primer sequence and repeated motif of each microsatellite.

For PCR screening of single flies, the head of each fly was macerated in a microfuge tube with a satay stick. DNA was extracted using a quick Chelex 100 (Bio-Rad) extraction method, based on the protocol of Walsh *et al*. (1991) and Moritz *et al*. (1992). Chelex preparations were stored at –20° for the long term, but were kept at 4°C for short periods of one or two months while experiments were in progress – no deterioration has been noted over years of storage. The remaining body and wings of each fly were stored individually at –70°C for future DNA preparations and/or for checking identifications.

Polymerase chain reaction primers were synthesized by Bresatec Ltd. One primer from each pair was synthesized with a fluorescent label HEX (hexachloro-fluorescein) at the 5′ end. The 10 μl PCR reactions contained 1× *Tth* plus buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.15 μM each primer, 0.165 U *Tth* plus DNA polymerase (Biotech International Limited) and 3 μl of a Chelex DNA supernatant. Polymerase chain reactions were set up in 96-well plates and carried out in a Hybaid Omnigene Thermal Cycler, under the following conditions: 1 cycle of 94°C for 3 min; 30 cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 50 s; 1 cycle of 72°C for 5 min. The PCR products were diluted one third with 80% deionized formamide containing 50:1 bromphenol-blue, denatured at 95°C for 3 min, then placed in ice. The diluted samples (1.5 μl) were electrophoresed in an ultra-thin 6% polyacrylamide gel, on a Corbett Automated DNA Fragment analyser, at 43°C, 1400 volts for 1 h, with size standards of GENESCAN-350 TAMRA (Perkin Elmer) and standard microsatellite alleles. The laser-scanned gel image was analysed using ONE-Dscan (Scanalytics), one-dimensional electrophoresis analysis software. A computer program was developed to combine data files for single acrylamide gels into an Excel spreadsheet entered as alleles of each microsatellite for each individual fly.

Methods of statistical analysis

A considerable literature has accumulated on methods of analysis for microsatellite markers (e.g. Feldman *et al*., 1997). The geographical complexity of the data set, however, suggested that a more empirical analysis, based on simple χ^2 tests, would effectively define population subdivisions. Although initially it was believed that variation beyond that expected by chance would be routinely found, in practice the comparisons, both between samples within regions and between regions in different years, gave close to the expected chance variation, thereby justifying this form of analysis.

Results were pooled over different microsatellites by calculating χ^2 values for each microsatellite separately and then summing χ^2 values and degrees of freedom. Owing to the different numbers of allelic classes present in different times and districts, the number of degrees of freedom (n) varies from one test to the next. Therefore for a uniform comparison, the statistic $\sqrt{2}\chi^2 - \sqrt{(2n-1)}$, which is distributed approximately as a standard normal (Fisher & Yates, 1948), was used.

A χ^2 method was also used to test each microsatellite within each location for agreement with Hardy-Weinberg

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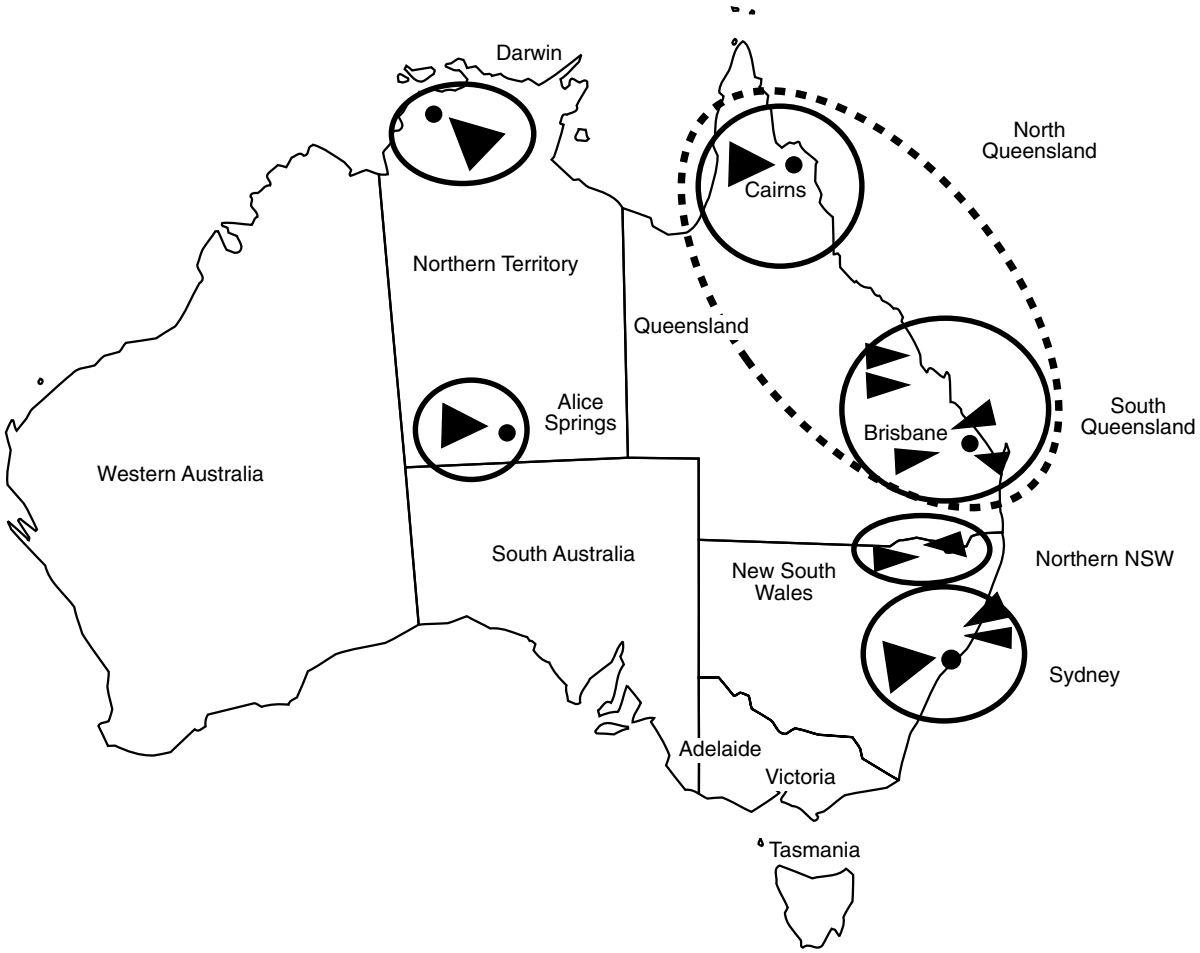

Fig. 1. Map showing locations of samples used in the microsatellite analysis of the Queensland fruit fly, *Bactrocera tryoni*. Locations marked with a small flag are represented by a single trap, while those with a large flag are represented by more than one trap. Regions within which samples are homogeneous (see text) are shown using ovals.

Table 1. Designation and sequence of microsatellite markers used in the present study of *Bactrocera tryoni*, showing original designations of Kinnear *et al*. (1998). Heterozygosities are estimated from the Queensland sample (see text).

New designation	Old designation	Simple sequence	Heterozygosity (%)
Bt10	11.7.1	$(CA)_3T(CA)_2A(CA)_7$	71.1
Bt11	11.2.5B	$(GT)_2AT(GT)_7$	74.1
Bt14	12.8.1B	$(GTT)_6$	61.1
Bt15	Bt15	$(TG)_8$	70.6
Bt17	3.3.5	$(TG)_2CG(TG)_9$	64.5
Bt32	Bt32	$(TG)_{11}$	89.7

frequencies. The test was actually a test of the overall frequency of homozygotes and heterozygotes, rather than a complete test of departure from Hardy-Weinberg equilibrium (Weir, 1996, Chapter 3). The test used the observed frequency at each locus, p_i , and calculated the expected frequency of homozygotes as

$$H = \sum_{i=1}^{k} p_i^2,$$

where k is the number of alleles at the locus, and the frequency of heterozygotes is 1 – H. A one-degree-of-freedom χ^2 was then calculated for each microsatellite in each region, and summed over regions to produce an overall χ^2 for each microsatellite. This test involved some approximation, although simulation showed that the mean of each calculated χ^2 statistic was close to its expectation of unity.

Genetic distances were calculated using the measure Φ^* of Latter (1973). This distance measure is equivalent to Fst when many populations are considered, but correctly estimates the divergence due to chance segregation for two or more populations.

A standard principal components analysis was used to

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picture regional and year differences. For this analysis, rare alleles were combined, thereby reducing a total of 124 alleles over the six microsatellites down to 49 allele classes. The two highest principal components, accounting for approximately 50% of the variation, were then calculated using these 49 frequencies. The principal components analysis does not make any allowance for the constraint that allele frequencies at each of the six loci must sum to unity.

Chromosomal localization of the microsatellites, by *in situ* hybridization and linkage mapping (J.T. Zhao, personal communication), showed that Bt14 and Bt32 were loosely linked, and that Bt10 and Bt15 were reasonably tightly linked, within 1cM or so. Significant levels of linkage disequilibrium were confirmed between the latter pair in some, but not all, populations. As a first approximation, the possibility of this association was ignored in the overall tests, in which χ^2 values from different loci were summed to test for divergence between populations.

Results

The complete data set, involving approximately 25,000 classifications, is too large for inclusion, but is available on request from the authors. Altogether over the five years, 2320 *B. tryoni* flies were analysed for the six microsatellites. Of these, 2075 flies (~90%) were successfully classified for all six markers (24,900 observations). For a conservative analysis, only these fully classified flies were used. Polymerase chain reaction products for each fly were scored from at least two gels and, in a proportion of samples, multiple PCR reactions were carried out, as controls for experimental error. There were no ambiguities in scoring the data (Kinnear *et al*., 1998). Heterozygosities are shown in table 1, and range from 60% to 90%, reflecting the fact that the six most variable of the 20 microsatellites isolated by Kinnear *et al*. (1998) were used in the study.

Testing within years – grouping into regions

Samples from each year were initially grouped into regions, based on *ad hoc* combinations of the closest traps. Data from successive samples were added to the analysis until it was clear that significantly heterogeneous samples were being pooled. It was found that the same samples could be pooled in each of the five years, thereby providing a strong justification for this procedure.

Regions defined by pooling homogeneous samples are shown in fig. 1. Heterogeneity tests for variation between samples within regions in each year are presented in table 2. North and South Queensland were treated separately for this analysis because of the large distance involved between the two sampling regions. The region described as Northern New South Wales is more accurately designated as the New South Wales Northern Tablelands region, an inland region characterized by high summer and low winter temperatures.

The results indicated a high uniformity of samples within regions. Two significant values at the 5% level were expected by chance in 42 comparisons. The test for overall significance summed over all regions and years was also not significant at the 5% level (snd = 1.86, $0.05 < P < 0.10$). Since the table was based on nearly 25,000 observations, the lack of significance indicated that differentiation within any of the designated regions must be minor.

The North and South Queensland samples were next tested against each other (table 3). The agreement between the two regions for each year and for the totals showed that there was no evidence for differentiation within the primary range of the species, despite the large distances involved. In further analyses, therefore, the two areas were combined.

Agreement with Hardy-Weinberg expectations

Chi-square values with one degree of freedom, testing for agreement of heterozygosity with Hardy-Weinberg expectations, are given in table 4. Values were close to expectation except for one microsatellite, Bt15, which showed a highly significant excess of homozygotes compared to expectation in three regions. The fact that only one locus deviated from expectation indicated that the discrepancy was likely to be due to a property of that locus, rather than due to population structure which should affect all loci equally.

The obvious cause of an apparent excess of homozygotes is the presence of one or more null alleles.

Table 2. Heterogeneity between *Bactrocera tryoni* samples within regions for each year, as tested by the standard normal deviate.

	1994	1995	1996	1997	1998	Total
North Queensland	1.09	1.14	–0.11	–0.55	–0.57	0.31
South Queensland	–1.20	–0.40	1.63	1.44	1.20	1.00
Northern NSW	0.50	0.31	1.70	–0.74	1.56	1.41
Sydney	2.06*	0.33	–0.42	–1.68	0.41	0.36
Darwin	–0.26	2.68*	1.51	0.97	–0.90	1.72
Alice Springs	0.63	–1.29	–0.21	0.02	0.11	–0.40
Total	0.50	1.23	1.57	–0.01	0.99	1.86

* (> 1.96) Significant at the 5% level.

Table 3. Test for heterogeneity between North and South Queensland *Bactrocera tryoni*, showing homogeneity of the regions in all years.

	1994	1995	1996	1997	1998	Total
χ^2	67.68	73.62	53.17	62.72	68.44	325.63
d.f.	76	58	60.00	58	66	318
N	2316	2256	1980	2100	2100	10752
s.n.d.	–0.65	1.41	–0.60	0.48	0.25	0.32

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Table 4. χ^2 testing for agreement of homozygosity in *Bactrocera tryoni* populations with random mating expectations.

	Bt10	Bt11	Bt14	Bt15	Bt17	Bt32
Queensland	0.47	0.77	0.77	32.14***	0.10	0.04
Northern NSW	3.22	0.73	2.84	11.32***	0.03	0.18
Sydney	0.93	1.43	0.87	13.37***	0.24	0.09
Darwin	0.51	0.00	0.10	0.09	0.59	0.13
Alice Springs	0.13	0.34	0.26	0.69	0.01	0.75
Total	5.26	3.27	4.84	57.61***	0.97	1.19

*** Significant at the 0.1% level.

The frequency of null alleles can be estimated from the deficiency of heterozygotes. The frequency of heterozygotes can be seen to be reduced compared to its expectation in the absence of null alleles by the factor $(1 - q)^2$, where q is the frequency of null alleles. This allows an estimate of q, which in the Queensland, Northern New South Wales and Sydney samples was 1.8%, 2.6% and 1.6% respectively. The frequency of null alleles appeared to be sufficiently low to allow the Bt15 results to be used with those from other loci.

The apparent absence of null alleles in the Darwin and Alice Springs populations is of interest. The result for Alice Springs is in line with the findings on rare alleles given below, showing that this population lacks variability compared to the eastern populations.

Testing between years

Given the homogeneity of samples within regions, the most powerful test for heterogeneity between years is given by first combining data for regions and then testing for differences between years within regions (table 5). The data revealed overall heterogeneity at the 5% level. When individual regions were tested for heterogeneity between years, significance was found only in the Northern New South Wales Tablelands region at the 5% level. This heterogeneity is further investigated below.

Testing between regions

When heterogeneity χ^2 tests were carried out between regions, all tests involving Darwin or Alice Springs were significant at the 0.01% level in each of the five years. There were also clear differences in most years between the different eastern regions (table 6). The most striking exception to this was the lack of differentiation between Queensland and Northern New South Wales during the final two years of the study.

To give an overall picture of the differences in *B. tryoni* populations between regions, a principal components analysis was carried out. Figure 2 shows the two highest components. The major differentiation between Darwin, Alice Springs and the east coast regions is clearly illustrated. The differentiation of the remaining three regions is less marked. However, in agreement with the analysis shown in table 6, the Queensland and Sydney populations remained differentiated at all stages. The separation of the Northern New South Wales region from these two other regions is less clear. The more variable nature of this region is not surprising, given the heterogeneity between years. However the smaller size of the sample in this case (see table 5) also contributed to the greater variability.

As a final visual presentation for the three eastern regions, genetic distances were calculated between regions in different years (fig. 3). The genetic distances involving the Sydney region, including that between Queensland and Sydney, stayed approximately constant between years. However the genetic distances between the Northern New

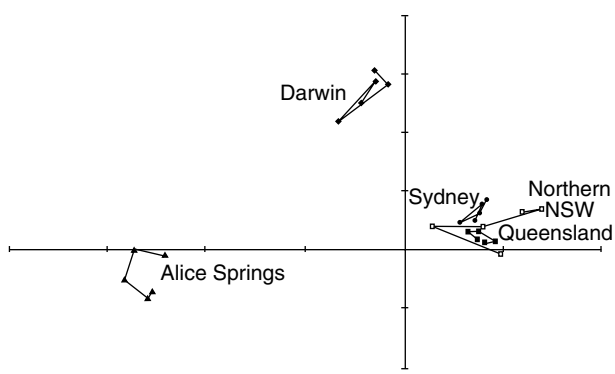

Fig. 2. Two-dimensional principal components analysis of the regional frequencies over time at six microsatellite loci in *Bactrocera tryoni*.

Table 5. Test for heterogeneity in Queensland fruit fly populations between years.

	χ^2	d.f.	N	s.n.d.
Queensland	399.24	368	10752	1.15
Northern NSW	293.27	236	2280	2.52*
Sydney	276.25	268	5520	0.38
Darwin	250.65	216	3492	1.63
Alice Springs	71.07	84	2856	–1.00
Total	1290.48	1172	24900	2.40*

* Significant at the 5% level.

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Table 6. Test for heterogeneity between eastern regions.

		1994	1995	1996	1997	1998
Sydney	χ^2	73.08	76.53	64.81	79.34	105.7
vs. Northern NSW	d.f.	53	52	47	52	51
	N	1380	1500	1464	1680	1776
	s.n.d.	1.84	2.22	1.74	2.44	4.48
	sig.		*		*	***
Sydney	χ^2	115.1	142.2	140.2	125.8	152.8
vs. Queensland	d.f.	77	65	63	65	72
	N	3120	3312	3024	3300	3516
	s.n.d.	2.8	5.5	5.56	4.5	5.52
	sig.	*	***	***	***	***
Northern NSW	χ^2	110.1	129.7	96.21	77.62	67.26
vs. Queensland	d.f.	76	65	64	60.00	67
	N	2892	2700	2400	2580	2460
	s.n.d.	2.55	4.75	2.6	1.55	0.06
	sig.	*	***	*		

*Significant at the 5% level.
***Significant at the 0.1% level.
Tests for heterogeneity involving Darwin and Alice Spring are not shown, but all were significant at the 0.1% level.

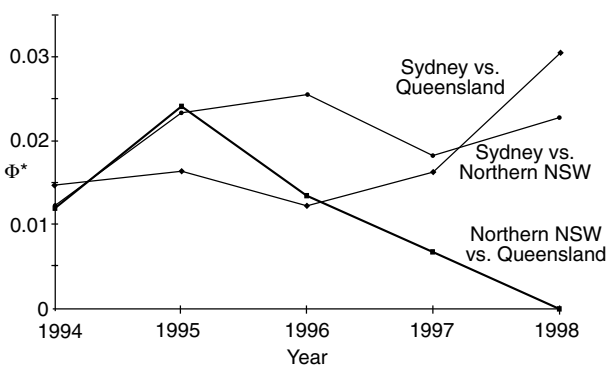

Fig. 3. Genetic distances (Φ* of Latter, 1973) between east coast populations of *Bactrocera tryoni* over five years of sampling, showing the convergence of the Northern New South Wales and Queensland samples over the last two years.

South Wales and Queensland regions decreased, particularly over the final two years of the sample. The significant heterogeneity between years for the Northern New South Wales region indicated that the change occurred in that region rather than in Queensland. Such a change is consistent with the sizes of the populations, and suggests that the more marginal Northern New South Wales population was substantially replaced by flies from Queensland in the final two years of the study.

The Alice Springs population

The data for Alice Springs showed a lack of variability compared to the other populations. Table 7 summarizes the number of occurrences of alleles occurring at an overall frequency of less than 2%. Alice Springs stands out in this analysis as having markedly fewer rare alleles than the other regions, indicating that the population has been through an extreme bottleneck (see Luikart *et al*., 1998). It seems probable that the population was founded on a single occasion from a limited number of founding individuals, and has since received no immigrants.

Discussion

Most of the conclusions in the paper come from heterogeneity χ^2 analyses. This form of analysis has become less common in the literature on population structure than analyses based on genetic distance methods. Our contention is that, where χ^2 analyses are possible, they are more revealing than analyses which do not take into account the

Table 7. Observed numbers of rare alleles in *Bactrocera tryoni*, showing the loss of variability in flies sampled from Alice Springs.

	Queensland	Northern NSW	Sydney	Darwin	Alice Springs	Total
Bt10	76	14	16	34	0	140
Bt11	64	15	20	13	1	113
Bt14	12	4	1	1	0	18
Bt15	43	9	29	5	0	86
Bt17	30	6	12	5	0	53
Bt32	187	24	31	22	0	264
Sum	412	72	109	80	1	674
Total sample	10752	2280	5520	3492	2856	24900
Per cent	3.8	3.2	2.0	2.3	0.0	2.7

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expected variability implied by the size of the samples, as also argued by Weir (1996, p. 167).

The close agreement of χ^2 with its number of degrees of freedom (tables 2 and 3) shows that the variability between samples was precisely as expected with samples from the same population. The estimate of genetic distances between samples within such regions is not significantly different from zero. Furthermore, the data reveal something about the sampling process. If, for example, flies entering traps tend to be from the same family, rather than being randomly sampled individuals from the population, increased values of χ^2 would be expected.

Such analyses are, however, only possible in organisms where there is limited change in frequencies, either temporally or spatially. It is the apparently homogeneous nature of the endemic populations, presumably a property of their large size, which make this analysis possible. By contrast, it has been found (manuscript in preparation) that outbreak populations are not amenable to a similar form of analysis, since heterogeneity between different samples is routinely found.

The endemic distribution

The results of the present study suggest that the distribution of *B. tryoni* in Australia may have a simple underlying pattern. As a first hypothesis, it may be postulated that there is a large 'core distribution', and that flies outside this range are genetically differentiated. For the sake of simplicity, and in view of the common name of the fly, it is convenient to define 'Queensland' as the core region of the fly.

The general picture is one of stability – a surprising stability given the high capacity for dispersal (see review by Meats, 1998). *Bactrocera tryoni* flies sampled from the same region were homogeneous in their microsatellite allele frequency distribution over the entire five year period. The only exception to this picture was the example of the population on the New South Wales Northern Tablelands over the last two years of the study. For the Sydney population, and perhaps for those in more southerly regions, this stability would suggest that current levels of migration from the core distribution of the fly in Queensland are not important in determining the typical microsatellite frequencies of populations.

The results indicate that little or no differentiation has occurred within Queensland. Presumably, despite the distances involved, migration must occur at a rate sufficient to overcome any tendency for regional differentiation, although such migration need not be at more than a reasonably small rate to ensure homogeneity (Wright, 1931). In this part of the range, flies are present in abundance at all times of the year, and breeding can occur year round (Meats & Khoo, 1976; Fletcher, 1989; Meats, 1989). Differentiation has, however, occurred in the more southerly parts of the continuous east coast range, and between the east coast and Northern Territory populations.

The differentiation is greatest between the two Northern Territory populations and between the east coast and Northern Territory populations. The geographic distances in these cases are, however, sufficiently large to minimize the likelihood of continuing migration. For the Alice Springs population, the lack of migration is clear (see results summarized in table 7). For the Darwin population, the situation is complicated by possible introgression of genes from the sibling species *B. aquilonis* (R.A.I. Drew, personal communication) during the founding of the population there.

The adaptation hypothesis

A striking result from the analysis was that differentiation has demonstrably occurred between the continuously-distributed Queensland and Sydney populations. This raises the possibility that adaptation plays a role in maintaining the differentiation. Attention is restricted here to these two populations on the east coast of Australia, because of the limited possibilities for migration to the Northern Territory, the strong evidence for a major founder effect in the Alice Springs population, and the uncertain level of introgression of *B. aquilonis* genes in the Darwin population.

On the east coast, there is clearly a substantial potential for migration, both aided and unaided by humans. A major effort is made to impede the spread of the flies in more southerly regions of eastern Australia (see Introduction). However, little effort is made to impede the flow between Queensland and the more northerly areas of New South Wales, including Sydney. The homogeneity of flies within Queensland argues for a potentially important effect of migration. The observation of a 'take-over' of New South Wales Northern Tablelands populations by the Queensland allele frequency distribution in 1997 and 1998 also shows the potential homogenizing effect of migration. Even with this population, however, it is perhaps more significant that differentiation had built up in 1994–1996 than that the differentiation appears to have been lost subsequently.

Despite the potential for migration, and the fact that the Queensland populations are much more abundant, there is no evidence that the population in the Sydney region has been affected by gene flow. Five years ought to be an adequate period to detect the influence of the Queensland population, should this be occurring. Overall, therefore, a likely reason why no effects of migration are detected is because flies from Queensland are not adapted to southern climates.

There seems little doubt that the potential for adaptation exists. Flies in south-east regions are under considerable selection pressure to increase cold tolerance and capacity for adults and pupae to survive the winter. Meats (1981, 1987) and O'Loughlin *et al*. (1984) have shown that the spread of the fly in the colder regions is limited by the ability of adults to survive that part of the year when daily maximum temperatures prevail below 20°C and reproduction is not possible. Thus, in southern Australia, the ability to survive frosts or the acute affects of cold is not important, except where frosts go below –2°C. The ability to survive the 'breeding gap' is critical. This 'breeding gap' varies from zero in coastal Queensland to several months in southern Victoria.

A balance between selection and migration may therefore be postulated, leading to a cline in the frequency of certain genes which affect the ability to survive and reproduce in southern regions. Although it is unlikely that the microsatellite loci are directly implicated in such clines, they could serve as markers for particular chromosomal regions which have differentiated during the adaptation process. Parenthetically, as argued following Wright (1931), it is possible that migration from the more numerous

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populations in Queensland has been beneficial in the long term in preventing the flies from evolving an even more successful cold-tolerant southern race. However, despite such migration, adaptation could have allowed some form of long-term differentiation.

Implications for control

Considerable efforts are made to achieve control of populations outside the core distribution of the fly. The status of designated fly-free areas is maintained by responding to spot outbreaks, several of which may occur in a season in some years. Only limited attempts are made to limit the Sydney and other New South Wales populations. It would perhaps be better to take a broader view of the distribution. A long-term strategy for control could be based on the likelihood that all populations outside of Queensland are genetically isolated, at least to some extent, from the core population, and that spot infestations that occur in fly-free areas are more likely to originate from adjacent areas than from the much more numerous northern population. Under this view, adjacent populations would be susceptible to an eradication strategy. Once eradication had been achieved, it would be difficult for the fly to re-invade from the core distribution in Queensland. In conclusion, definition of fruit fly population subdivisions may greatly facilitate management.

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