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Geographical variation, population structure and gene flow between populations of *Chrysophtharta agricola* (Coleoptera: Chrysomelidae), a pest of Australian eucalypt plantations

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Abstract

Chrysophtharta agricola (Chapuis) is a pest of commercial eucalypt plantations in Tasmania and Victoria. Vagility of pest populations may result in difficulty predicting temporal and spatial pest outbreaks, and influence genetic resistance to chemical control. Gene flow in this pest species was estimated to assess predicability of attack, the potential efficacy of natural enemies, and the likelihood of resistance build-up. Ten geographic populations of ***C. agricola*** (six from Tasmania, one from the Australian Capital Territory, one from New South Wales and two from Victoria) were examined for genetic variation and gene flow using cellulose acetate allozyme electrophoresis. Six enzyme systems (PGI, PGD, PGM, IDH, HEX and MPI) were consistently polymorphic and scorable and were used to quantify estimated gene flow between populations. FST values and analysis of molecular variance indicated that gene flow was restricted between populations. ***Chrysophtharta agricola*** exhibited high levels of heterozygosity, probably because of high allelic diversity, and because all loci examined were polymorphic. The southern-most population was the most genetically different to other Tasmanian populations, and may also have been the most recently colonized. Limited gene flow implies that outbreaks of ***C. agricola*** should be spatially predictable and populations susceptible to control by natural enemies. Our results also imply that genetic resistance to chemical control may occur under frequent application of insecticide. However, testing population movement between plantations and native forest also needs to be conducted to assess gene flow between forest types.

Introduction

The balance between genetic drift, natural selection and gene flow determines the genetic structure of populations. Stochastic forces including genetic drift and founder effects lead to genetic differentiation of populations, while natural selection may increase or decrease genetic differentiation and gene flow tends to homogenize the genetic structure of populations (Peterson & Denno, 1998). Gene flow in natural insect populations is restricted by dispersal, age, host-plant patchiness, and host-plant longevity; where no such isolating factors act, homogeneity of genetic diversity results (Mopper, 1996). For example, despite mountains providing very effective barriers to gene flow in chrysomelid beetles (Knoll *et al.*, 1996), different populations of *Chrysophtharta*

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bimaculata (Olivier) (Coleoptera: Chrysomelidae), a serious pest of eucalypt plantations in Tasmania, are genetically indistinguishable from a single, interbreeding population (Congdon *et al.*, 1997).

In pest species, gene flow is important in the evolution of insecticide resistance and adaptation to local environmental conditions. Understanding gene flow is important because a species' pest potential is partially a consequence of its ability to invade and reproduce in novel habitats (Daly, 1989), and to determine the spatial scale at which management practices should be implemented (Congdon *et al.*, 1997). Gene flow can be indirectly estimated using allozyme electrophoresis, a technique that provides basic genetic and biochemical information and quantifies the effect of gene flow on the genetic composition of different populations by examining the non-random spatial association of neutral alleles (Richardson *et al.*, 1986; Rowell-Rahier, 1992; Costa, 1998). Many herbivorous insects show significant genetic variation at small spatial scales, possibly because of host plant patchiness (Rank, 1992). Patchiness or sub-division of suitable habitat is largely responsible for the semi-isolation of populations, which thereby comprise an array of local breeding populations potentially connected by gene flow (Wade & McCauley, 1988; McCauley, 1993).

Highly vagile pest populations may present temporal and spatial difficulties in predicting outbreaks and may be less susceptible to control by natural enemies (Drake, 1998). Furthermore, genetic resistance to chemical control can occur more rapidly in populations where gene flow is limited or absent. Thus, determining the mobility and dispersal of pests using allozyme electrophoresis may provide information on predictability of attack, the potential efficacy of natural enemies and the likelihood of insecticide resistance build-up. However, for most agricultural pest species, spatial variation in pesticide application, plant genotypes and biocontrol agents are likely to result in local adaptation only under extreme selective differences (Peterson & Denno, 1998). Agricultural insect pests typically show high levels of gene flow relative to species occurring in natural habitats, although species' endemism does not influence gene flow (Peterson & Denno, 1998).

Congdon *et al*. (1997) conducted a large electrophoretic study to test for genetic variability in *C. bimaculata* populations throughout Tasmania, comparing different geographic regions and different host eucalypt species. *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae), like *C. bimaculata*, is pest of eucalypt plantations in Tasmania (de Little, 1989; Elliott *et al.*, 1998), and Victoria (Elliott *et al.*, 1998; Collett, 2001). However, *C. bimaculata* forms large aggregations of thousands of individuals (Howlett *et al.*, 2001), often observed flying as loose aggregations (Clarke *et al.*, 1997), while *C. agricola* forms 'moderately dense aggregations' (Howlett, 2000), and is not known to undergo such large-scale migratory flights. Because gene flow is enhanced by mobility (Peterson & Denno, 1998), *C. bimaculata* might be expected to be more genetically homogeneous than *C. agricola*, and this hypothesis is tested within Tasmania. Furthermore, Bass Strait is predicted to provide a barrier to gene flow (Cranston & Naumann, 1991) and this is tested by comparing the genetic structure of *C. agricola* populations between mainland Australia and Tasmania. Here, the results of allozyme electrophoresis to examine genetic population structure of *C. agricola* using ten geographic populations from Tasmania and mainland Australia are reported.

Materials and methods

Chrysophtharta agricola were collected from six localities throughout Tasmania, and four from mainland Australia (fig. 1). Collections in mainland Australia were made from native forest, whereas collections from Tasmania represented populations resident in commercial eucalypt plantations. Up to 48 adults were collected from each site; however, for sites at which large numbers of adults were not present, egg and larval batches were collected (table 1). Such batches were held individually in vials or Petri dishes and reared to adulthood in the laboratory. Only one adult from each batch per site reared thus was used in electrophoresis. Adults obtained in this manner, and those collected from the field, were starved for 24 h, then killed and stored in a –70°C freezer until analysis.

Frozen beetles were bisected longitudinally using a scalpel blade that was washed with warm water and detergent, and rinsed twice in distilled water between uses. One half of each beetle was ground in 100 µl of grinding buffer (1.22 g Trizma base; 0.185 g EDTA-free acid; 2.68 g

Table 1. Sites and hosts from which *Chrysophtharta agricola* were collected for electrophoretic study.

Site	Code	Host species	Collected as (total frozen)	Males	Females
Picadilly Circus, Australian Capital Territory	PIC	*Eucalyptus dalrympleana* Maiden	26A, 22 reared from E, L (48)	24	24
Jindabyne-Thredbo Road, New South Wales	JIN	*E. dalrympleana*	7A, 29 reared from E, L (36)	15	21
Mt Buller, Victoria	BUL	*E. viminalis* Labill.	21A, 27 reared from E, L (48)	20	28
Marysville, Victoria	MAR	*E. viminalis*	10A, 23 reared from E, L (33)	15	18
Ridgely, Tasmania	RGY	*E. nitens* (Deane & Maiden) Maiden	48A (48)	18	30
Scottsdale, Tasmania	SCO	*E. globulus* Labill.	1A, 39 reared from E, L (40)	15	25
Frankford, Tasmania	FRA	*E. nitens*	48A (48)	24	24
Florentine Valley, Tasmania	FLO	*E. nitens*	48A (48)	20	28
Ellendale, Tasmania	ELL	*E. nitens*	48A (48)	22	26
Geeveston, Tasmania	GEE	*E. globulus*	33 reared from E, L (33)	15	18

E, eggs; L, larvae; A, adults.

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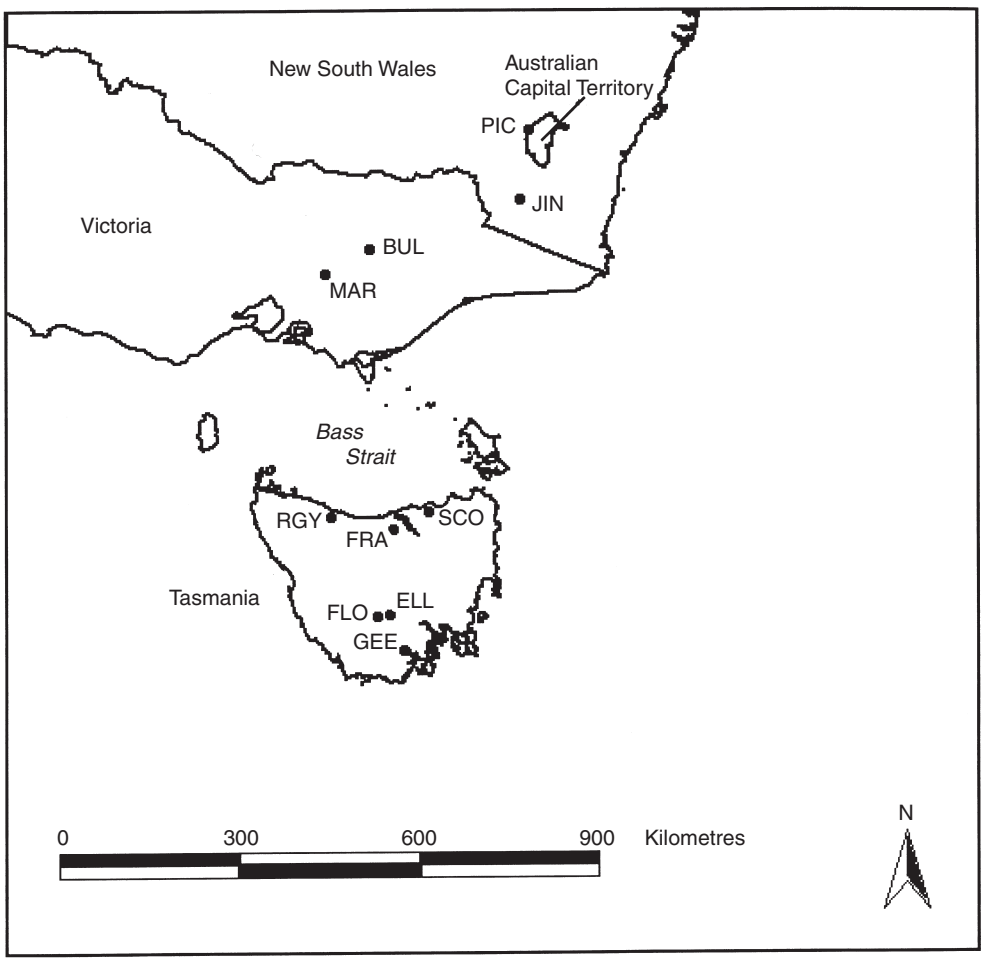

Fig. 1. Map of the south-eastern Australian mainland and Tasmania showing the sites from which *Chrysophtharta agricola* was collected for allozyme electrophoresis. Sites are coded as in table 1.

NH_4Cl; 9.9 g glucose in 500 ml distilled water) in a 1.5 ml Eppendorf tube. The homogenates were centrifuged in an Eppendorf Centrifuge 5417R at 14,000 rpm for 6 min at 4°C. Upon removal, tubes were stored in ice, and 10 μl of the supernatant was pipetted into wells of a Helena Laboratories sample plate, also held on ice. Samples were run on cellulose acetate plates using either Tris-citrate pH 7.0, 75 mM running buffer (45.4 g Tris; 25.3 g citric acid in 5 l distilled water) or Tris-glycine pH 8.5, 50 mM running buffer (15 g Tris, 64.4 g glycine in 5 l distilled water). Plates in TC buffer were run at 150 V, 5 mA per plate; plates in TG buffer were run at 200 V, 1.5 mA per plate. Hexokinase (HEX (EC code 2.7.1.1)) and mannose-phosphate isomerase (MPI (EC 5.3.1.8)) were run in TG buffer for 1 h, and phosphoglucosemutase (PGM (EC 2.7.5.1)) was run in TG for 45 min. Isocitrate dehydrogenase (IDH (EC 1.1.1.42)), 6-phosphoglucose dehydrogenase (PGD (EC 1.1.1.44)), and phosphoglucose isomerase (PGI (EC 5.3.1.9) were run in TC buffer for 2 h. Enzyme stain procedures were those described by Hebert & Beaton (1989). Between 10 and 12 individuals were run on each cellulose acetate plate, with individuals from each population run simultaneously for each enzyme. Afterwards, several line-up plates were conducted for each enzyme, using individuals from different populations to double-check allele band positions between individuals and populations.

An average of 41 beetles (range 23–48) was scored for each site, for each of six enzymes. The mean number of alleles per locus and observed levels of heterozygosity were calculated for each population, and the mean and effective numbers of alleles, and heterozygosities were calculated for each locus. For each of these measurements, significant differences were detected using ANOVA, with post-hoc differences determined using Fisher's least significant difference (LSD) test, with $\alpha = 0.05$, in SYSTAT 10 (SPSS Inc, 2000).

Genetic data were analysed using GenAlEx (Peakall & Smouse, 2001) and Genetic Data Analysis (Lewis & Zaykin, 2001). F_{ST}, and F_{IS} values (Wright, 1931), computed using all loci combined, comparing sites and regions were estimated using Genetic Data Analysis, and 95% confidence intervals for these values were determined using bootstrap analysis with 10,000 permutations. F_{ST} measures the genetic variation between populations (Hsiao, 1989). F_{ST} values that are significantly greater than zero imply that distinct population structure exists between populations. F_{ST} can also be used to estimate the mean-per-generation number of migrants exchanged among populations, Nm, where $Nm \cong (1-F_{ST})/4F_{ST}$ (Avise, 2000). This calculation assumes an island model of population structure, where a species is subdivided into populations of equal size N, all of which

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exchange alleles with equal probability (Wright, 1931). F_{IS} provides a measure of the degree of inbreeding in individuals relative to the population to which they belong (Giles & Goudet, 1997). F_{IS} values that do not differ significantly from zero imply that random mating occurs within populations. F_{IS} values were also computed for each site separately. *F*-statistics were also computed for each enzyme separately using GenAlEx. Analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) using 999 permutations was used to determine differences between regions (mainland Australia and Tasmania) and sites for each enzyme. Nei (1972) genetic distance was estimated in Genetic Data Analysis for all enzymes combined. Mantel correlations were performed using GenAlEx (999 permutations) for enzymes that showed significant genetic variation between populations to examine the relationship between linear geographic and linear genetic distance. The percentage of genetic variation between regions, among populations within regions and within populations was determined for each enzyme. The number of permutations performed was the maximum possible for each software package: 999 and 10 000 for GenAlEx and Genetic Data Analysis, respectively.

Results

All enzymes used were polymorphic: HEX comprised two loci with four and two alleles each. The other enzymes comprised one locus each: IDH and PGM had four alleles, PGD and PGI had three alleles, and MPI had six alleles. The mean number of alleles per locus did not differ significantly between regions: mainland Australian sites had a mean of ± s.e. 3.4 ± 0.2 (range 3.3–3.6) alleles per locus for the six enzymes examined, and Tasmania had 3.3 ± 0.2 alleles per locus (range 3.0–3.6) (t-test, $t_{68} = 0.4$, $P = 0.7$), or between populations within regions (ANOVA, $F_{9,60} = 0.23$, $P = 0.9$). Likewise, there was no significant difference between the average observed levels of heterozygosity between regions (t-test, $t_{68} = 0.7$, $P = 0.5$) or between populations (ANOVA, $F_{9,60} = 0.14$, $P = 0.9$). Mean ± s.e. heterozygosity over the six enzymes examined was 0.50 ± 0.04 (range 0.40–0.52) for Tasmania, and 0.50 ± 0.05 (range 0.49–0.55) for mainland Australia.

The averages for actual and effective number of alleles and average observed heterozygosity per locus was calculated (table 2). The actual and effective numbers of alleles were significantly different between loci (ANOVAs, $F_{6,63} = 70.2$, $P < 0.001$; $F_{6,63} = 55.4$, $P < 0.001$, respectively), with *Mpi* and *Idh* the only enzymes that were consistently higher than all others for both parameters (Fishers LSD post-hoc tests, table 2). Mean heterozygosity was also significantly different between loci (ANOVA, $F_{6,63} = 80.95$, $P < 0.001$). *Idh* had the significantly highest level of heterozygosity, whilst *Pgi* had the significantly lowest level of heterozygosity (Fishers LSD post-hoc test, table 2).

F_{ST} values calculated using allozymes combined (table 3) were significantly greater than zero, and F_{ST} values calculated for allozymes separately (table 2) were all positive (mean = 0.04), indicating that significant population structure of *C. agricola* exists between populations as a result of restricted gene flow. F_{IS} values were not significantly different from zero in both analyses with sites combined (mean = –0.02), and were likewise not significantly different from zero for sites examined separately (table 3).

Furthermore, the results of AMOVA (table 2) illustrate significant differences between populations. Analysing each

Table 2. The average (± s.e.) number of alleles, effective number of alleles, heterozygosity, F_{ST} and F_{IS} values for each locus over ten geographic populations of *Chrysophtharta agricola*. The results of analysis of molecular variance (AMOVA) for these seven loci for at regional (mainland Australia and Tasmania) and population levels, and results of Mantel correlation for each are also presented, as well as the % genetic variation between regions, populations within regions and between individuals within populations.

	Pgd	*Pgm*	*Hex 1*	*Hex 2*	*Idh*	*Mpi*	*Pgi*
Number of alleles	2.8 ± 0.1 de	3.3 ± 0.2 c	3.1 ± 0.2 ce	2.0 ± 0.0 f	3.9 ± 0.1 b	5.6 ± 0.2 a	2.5 ± 0.2 d
Effective number of alleles	1.5 ± 0.1 d	2.0 ± 0.1 c	2.3 ± 0.1 c	1.7 ± 0.1 d	2.9 ± 0.1 b	3.4 ± 0.23 a	1.1 ± 0.03 e
Heterozygosity	0.3 ± 0.03 d	0.5 ± 0.1 c	0.5 ± 0.03 c	0.3 ± 0.04 d	0.96 ± 0.02 a	0.8 ± 0.04 b	0.1 ± 0.02 e
F_{ST}	0.08	0.04	0.04	0.02	0.01	0.05	0.02
F_{IS}	0.14	0.07	0.04	0.19	–0.45	–0.08	–0.02
AMOVA							
Regions (Mainland and Tasmania)	$P = 1$, Phi = 0	$P = 0.03$, Phi = 0.02	$P = 1$, Phi = 0.00	$P = 0.06$, Phi = 0.00	$P = 0.01$, Phi = 0.02	$P = 0.001$, Phi = 0.07	$P = 0.001$, Phi = 0.07
Populations within regions	$P = 0.001$, Phi = 0.1	$P = 0.001$, Phi = 0.05	$P = 0.001$, Phi = 0.06	$P = 0.1$, Phi = 0.02	$P = 0.05$, Phi = 0.01	P = 0.001, Phi = 0.06	P = 0.8, Phi = 0
Mantel correlation	Rxy = –0.03 $P = 0.98$	Rxy = 0.01 $P = 0.17$	Rxy = –0.01 $P = 0.78$	Rxy = –0.01 $P = 0.66$	Rxy = 0.01 $P = 0.21$	Rxy = 0.11 $P = 0.001$	Rxy = 0.09 $P = 0.003$
% variation							
Between regions	0	2	0	0	2	7	7
Among populations	10	5	6	2	1	6	0
Within populations	90	93	94	98	97	87	93
n	421	426	411	412	422	417	389

Different letters for each mean in the first three rows represent significant differences at $P < 0.05$. *n* is the number of individuals tested for each locus.

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Table 3. F_{ST} and F_{IS} values (95% confidence limits in parentheses) across seven loci for *Chrysophtharta agricola* populations from different regions (Tasmania and mainland Australia), and F_{IS} values for populations within regions.

Site/region	F_{ST}	F_{IS}
Tasmania vs. Mainland	0.02 (0.005–0.04)	−0.02 (−0.206–0.141)
Tasmanian sites only	0.03 (0.01–0.04)	−0.01 (−0.21–0.174)
Geeveston		0.01 (−0.26–0.27)
Scottsdale		0.02 (−0.14–0.24)
Frankford		−0.05 (−0.21–0.12)
Florentine		−0.006 (−0.3–0.25)
Ellendale		0.04 (−0.2–0.26)
Ridgley		−0.07 (−0.26–0.11)
Mainland sites only	0.02 (0.003–0.04)	−0.09 (−0.25–0.08)
Picadilly Circus		−0.00 (−0.19–0.22)
Mount Buller		−0.12 (−0.3–0.07)
Marysville		−0.17 (−0.36–0.01)
Jindabyne		−0.11 (−0.27–0.07)
All sites combined	0.03 (0.02–0.05)	−0.04 (−0.222–0.124)

locus separately showed significant differences at four loci (*Pgm*, *Idh*, *Mpi* and *Pgi*) between mainland Australia and Tasmania, and at four loci (*Pgd*, *Pgm*, *Hex1* and *Mpi*) between populations within regions. These differences between regions, and between populations within regions suggest that there is restricted gene flow at both spatial scales, resulting in differences in the population structure of *C. agricola*. For two enzymes (*Mpi* and *Pgi*) at which significant differences were observed, Mantel correlation showed that geographical distance was correlated with genetic distance, but genetic differences between the remaining loci were not explained by geographical distance.

The proportion of genetic variation between regions, among populations within regions, and within populations was calculated for each enzyme (table 2). The greatest amount of genetic variation was attributable to between individuals within populations, while the greatest contribution of regional variation (7%) was for *Mpi* and *Pgi*, and the greatest within-population variation occurred at *Pgd* (~10%). Genetic distance estimates (Nei, 1972) and shortest geographical distance for each pair of sites are presented in table 4. These data show that *C. agricola* from Mount Buller exhibited the highest genetic distance estimates, and thus, differed most significantly from other populations. The Geeveston population differed most significantly from other Tasmanian populations (fig. 2). However, removal of the Geeveston data from analysis of Tasmanian populations still showed significant population structure between remaining populations (F_{ST} = 0.02, 95% C.I. 0.005–0.034; F_{IS} = –0.014, 95%C.I. –0.21–0.175), suggesting that significant mixing between populations within Tasmania does not occur.

Discussion

Chrysophtharta bimaculata is a highly vagile pest species which undergoes flights consisting of large numbers of beetles (Clarke *et al.*, 1997; Howlett, 2000), and whose host plants are continuously distributed across the central region of Tasmania (Congdon *et al.*, 1997). It is therefore not surprising that the species exhibits little genetic differentiation between sub-populations. *Chrysophtharta agricola* has not been recorded or observed undergoing such large-scale flight patterns, and shows a stronger level of genetic differentiation between sub-populations, despite a continuous geographical distribution of suitable host species (authors' unpublished data). Both species occur in young plantations

Table 4. Distance matrix for all sites.

	GEE	SCO	FRA	FLO	ELL	RGY	PIC	BUL	MAR	JIN
GEE		229	203	70	62	243	883	677	518	758
SCO	0.051		67	185	179	143	655	463	303	531
FRA	0.036	0.023		146	145	82	689	474	315	564
FLO	0.05	0.0251	0.022		17	175	834	617	460	709
ELL	0.055	0.012	0.033	0.015		182	831	618	460	706
RGY	0.045	0.041	0.035	0.021	0.038		695	454	304	570
PIC	0.031	0.057	0.037	0.036	0.046	0.031		289	396	125
BUL	0.050	0.053	0.057	0.070	0.076	0.064	0.037		160	195
MAR	0.031	0.038	0.050	0.037	0.036	0.025	0.024	0.039		275
JIN	0.025	0.051	0.047	0.039	0.042	0.028	0.010	0.039	0.008	

Geographical distance (km) between pairs of sites is presented above the diagonal, genetic distance (Nei, 1972) between *Chrysophtharta agricola* populations is presented below the diagonal. Populations are coded as in table 1.

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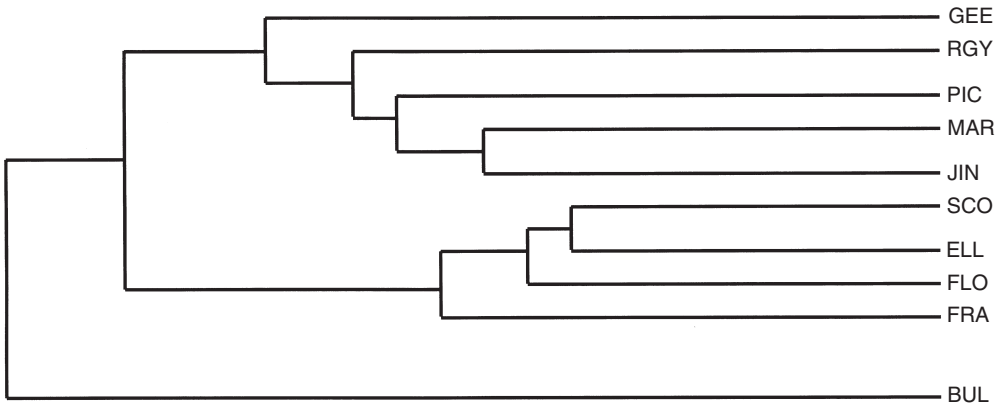

Fig. 2. Dendogram for *Chrysophtharta agricola* populations generated in Genetic Data Analysis, based on genetic distance/identity estimates. All nodes are significant at $P < 0.05$. Sites are coded as in table 1.

of *Eucalyptus nitens* (Deane & Maiden), but apparently do not compete for resources, with *C. bimaculata* restricted to adult foliage (de Little, 1989), and *C. agricola* more common on juvenile foliage (Elliott *et al.*, 1998; Nahrung & Allen, in press).

The *C. agricola* population collected from Geeveston (43°11′S) differed most significantly from other Tasmanian populations. Geeveston may represent an area colonized more recently by *C. agricola* with the introduction of eucalypt plantations in the south of the state in the 1990s (D. Bashford, Forestry Tasmania, personal communication). de Little's (1979) extensive survey did not locate any *C. agricola* further south than a latitude of 42°23′S, while Bashford (unpublished data) plotted its southerly distribution at 42°39′S in the 1980s, and its most-southerly collection record was 42°53′S in 1975 (T. Weir, Australian National Insect Collection, personal communication), about 50 km from Geeveston. The Geeveston population exhibited the lowest mean number of alleles and heterozygosity of the *C. agricola* populations in this study, and a low level of heterozygosity is consistent with a founder effect.

Chrysophtharta agricola had a higher level of heterozygosity and a higher mean number of alleles per locus than *C. bimaculata*, but we examined a larger proportion of polymorphic loci than did Congdon *et al.* (1997) for *C. bimaculata*. The high levels of heterozygosity observed here are the result of high allelic diversity: larger numbers of alleles would be expected to lead to high heterozygosity because of the increased number of possible allelic combinations. *F*-statistics and AMOVA suggest that *C. agricola* populations are genetically sub-divided between regions and populations. With the exception of the Geeveston population, the average Nei (1972) genetic distances between pairs of sites within regions was higher for inter-regional pairs than for intra-regional pairs (table 4). The population age-gene flow hypothesis (see Peterson & Denno, 1998) suggests that apparent gene flow is reduced among young populations compared to old populations: whether the high numbers of *C. agricola* in eucalypt plantations represent 'new' populations is arguable. Restricted gene flow might further be predicted from several aspects of *C. agricola*'s biology. Overlap in generations; adults and larvae sharing the same host plants; woody host plants; no mobile aggregations; males and females sharing overwintering habitat can each potentially result in restricted gene flow (Rank, 1992; Clarke *et al.*, 1997; Congdon *et al.*, 1997; Peterson & Denno, 1998). However, a normal sex ratio, multiple mating, and well-developed wings and mobility are traits that *C. agricola* possesses that can potentially lead to genetic mixing (Costa, 1998; Peterson & Denno, 1998). Peterson & Denno (1998) also reported that agricultural pest species are more likely to exhibit greater levels of gene flow than their counterparts in natural habitats.

Bass Strait is thought to provide a barrier to gene flow since it separated mainland Australia and Tasmania around 10,000 years ago (Cranston & Naumann, 1991), although some insects are documented crossing Bass Strait under favourable weather conditions (e.g. Drake *et al.*, 1981). There was a striking difference in elytral coloration between *C. agricola* from mainland Australia and from Tasmania which further suggests that gene flow is absent between these regions. However, the present results imply that migration (estimated at 12 individuals per generation, based on the relationship between F_{ST} and migration, where $Nm \cong (1-F_{ST})/4F_{ST}$ (Wright, 1931)) occurs between regions. Avise (2000), however, warned that this type of gene flow estimate fails to distinguish ongoing genetic exchange from the effects of historical associations among populations. Whether our F_{ST} values represent high contemporary gene flow, or past gene flow, or a mix of the two, is unclear. Furthermore, although complete isolation ($Nm < 1$) corresponds to $F_{ST} > 0.2$, many studies with $F_{ST} < 0.2$ but > 0 nevertheless conclude restricted gene flow (see Knoll *et al.*, 1996; Giles & Goudet, 1997). Additionally, F_{ST} and corresponding *Nm* estimates apply to an island model of population structure (Wright, 1931). While this model predicts that extremely low migration between populations is enough to prevent population differentiation through genetic drift, Haldane (1930) argued that natural selection within a population could lead to genetic differentiation despite gene flow occurring. However, the AMOVA results presented here also demonstrate restricted gene flow between populations within and between regions.

The population originating from Mount Buller was most different genetically to all other *C. agricola* populations (table 4, fig. 2) and also differed significantly from other populations in egg and larval parasitism rates (authors' unpublished

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data). Mountains can provide very effective barriers to gene flow in the Chrysomelidae (Knoll *et al*., 1996) and so the Snowy Ranges and Central Highlands of Victoria may prevent significant gene flow between *C. agricola* populations. Similarly, Tasmanian populations may be genetically differentiated because of gene flow restriction attributable to mountains and the clearing of native eucalypt forests across northern Tasmania (Congdon *et al*., 1997). Furthermore, the abundant resources provided by monospecific stands of hosts in eucalypt plantations, and the high numbers of beetles within them means that beetles do not need to disperse to locate suitable food, mates, or overwintering sites. Peterson & Denno (1998) found that insects that feed on woody host plants exhibit low levels of gene flow, probably because of the temporal persistence of woody plants.

Limited gene flow implies that outbreaks of *C. agricola* should be spatially predictable, and populations susceptible to control by natural enemies (*sensu* Drake, 1998). The latter has been observed in the field, with over 90% egg–pupal mortality recorded (authors' unpublished data). Although *C. agricola* shows limited gene flow between populations, and genetic resistance to chemical control may occur more rapidly under this scenario, we cannot conclude that this would be the case for *C. agricola*. Because populations originating in native forest in Tasmania were not sampled, there are no data available on estimates of beetle movement between native forest and plantations. Hence, whether resistance build-up might be likely to occur with regular application of insecticide in plantations cannot be predicted. Populations of *C. agricola* resident in plantations are much larger than those present in native forest (personal observations). Rapid population expansion under conditions of abundant resources, as in a plantation, may have an effect on the genetic structure of populations, similar to the population age-gene flow hypothesis described by Peterson & Denno (1998).

This study showed that *C. agricola* populations exhibit significant population structure that suggests random mating between individuals within populations, but not between populations. *F*-statistics and AMOVA results demonstrated significant differences between *C. agricola* populations, both between mainland Australia and Tasmania, and between populations within these regions.

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