Little geographic or host plant genetic variation in a *Chionochloa* (Poaceae) seed predator (Cecidomyiidae: undescribed species)

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Abstract: The grass genus *Chionochloa* in New Zealand exhibits a high degree of mast seeding synchronised across species and habitats. Masting appears to be maintained by a predator satiation mechanism involving three pre-dispersal seed- and flower-feeding insects. It is not clear how important each of the three insects is in favouring the masting strategy. An undescribed cecidomyiid fly (Diptera: Cecidomyiidae) may be particularly important, since its conspicuous larvae are found throughout the South Island of New Zealand on many *Chinochloa* species. Despite the wide distribution of the larvae, it is not clear whether they are conspecific. Since the species is undescribed and adults are rarely seen, there may be different species on different host plants or in different geographic areas. We used Inter Simple Sequence Repeats (ISSRs) to determine whether cecidomyiid larvae found in four different areas in the South Island and on four species of *Chionochloa* exhibited molecular variation consistent with the presence of a single species of fly. Cluster analysis using Unweighted Pair-Group Method using Arithmetic averages (UPGMA) based on 38 ISSR fragments showed no clusters based on either host plant or geography. Analyis of Molecular Variance (AMOVA) analyses showed statistically significant differentiation among both host populations and geographic populations, but most of the molecular variation was explained by individual variation within geographic regions and host-plant populations. Thus, the molecular variation in the cecidomyiid larvae suggests the presence of a single species of cecidomyiid. Our data, combined with previous population surveys, suggest that the cecidomyiid is the most widespread of *Chionochloa* seed predators and may provide the selective benefit for the synchronous flowering observed among different *Chionochloa* populations in New Zealand.

Keywords: Cecidomyiidae; *Chionochloa*; ISSR; mass flowering; mast seeding; predator satiation.

Introduction

Masting or mast seeding is the synchronous, highly variable seed production among years by a population of plants (Kelly, 1994). Masting may appear to be evolutionarily unstable, since years of little or no reproduction are lost opportunities for recruitment (Waller, 1979). Additionally, areas of dense offspring created by large influxes of propagules may contribute to intense intraspecific competition (Hett, 1971) or may attract more predators and pathogens than less-dense stands of plants (Root, 1973).

There are two categories of hypotheses used to explain the maintenance and/or evolution of the masting trait: (1) resource matching hypotheses and (2) economies of scale hypotheses (Norton and Kelly, 1988). Resource matching hypotheses were not supported by recent work involving literature surveys (Kelly and Sork, 2002) and individual-plant based models of masting (Rees *et al*., 2002). Most of the evidence to date supports two hypotheses under the economy of scale category – wind pollination (Nilsson and Wästljung, 1987; Norton and Kelly, 1988; Kelly *et al*., 2001) and predator satiation (Silvertown, 1980; Kelly and Sullivan, 1997; Kelly *et al*., 2000). In the case of wind pollination, masting serves to increase the long-term mean percentage of filled ovules since there are higher fertilisation rates in masting years than in non-masting years (Nilsson and Wästljung, 1987; Sork, 1993; Kelly *et al*., 2001).

Predator satiation occurs when a lower proportion of seed is damaged by seed predators during years of high reproductive effort than in years of low reproductive effort (Silvertown, 1980). There may also be a numerical response in the seed predators, where total predator numbers are reduced in years of low reproduction and are unable to rebound sufficiently to consume a large proportion of resources in mast years.

Alternation of low and high years of reproduction

may thus serve to keep average predator numbers low throughout many seasons (Kelly and Sullivan, 1997). The relative benefits of masting due to increased pollination efficiency or predator satiation have been investigated in a number of studies, with predator satiation appearing to be more important in some genera such as *Quercus* and *Chionochloa* (Sork, 1993; Kelly and Sullivan, 1997), and the relative benefits from wind pollination being greater in other genera such as *Betula* (Kelly *et al*., 2001).

The tussock grass genus *Chionochloa* in New Zealand benefits from masting by satiating its common seed predators (Kelly, 1994; Kelly and Sullivan, 1997; Kelly *et al*., 1992; Kelly *et al*., 2000; Kelly *et al*., 2001; McKone *et al*., 2001), but the identity of the seed predator responsible for the maintenance of masting is unknown. Three main seed predators may be involved in the maintenance of masting: a moth, *Megacraspedus calamogonus* (Lepidoptera: Gelichiidae); a Chloropid fly, *Diplotoxa similis* (Diptera: Chloropidae); and an undescribed cecidomyiid fly (Diptera: Cecidomyiidae) (White, 1975; McKone *et al*., 2001). The larval stages of these insects account for the majority of damage observed in *Chionochloa* inflorescences (White, 1975).

Chionochloa species have extremely high variation among years in the size of their seed crops and Kelly *et al*. (2000) argued this was probably due to the high levels of seed predation, and the fact that the biology of the seed predators may make them especially difficult to satiate. The relative contributions to the maintenance of masting by the three seed predators is unknown, but the cecidomyiid seems likely to be the most resistant to satiation, due to its apparent higher fecundity and greater mobility than *Diplotoxa similis*, and its possible extended and predictive diapause (Kelly *et al*., 2000; McKone *et al*., 2001), which would allow it to delay maturation between mast events. The cecidomyiid also has the widest recorded geographic and host ranges, being present at almost every site and in every *Chionochloa* species which has been searched at the appropriate time of year, whereas *Diplotoxa* and *Megacraspedus* have been found in fewer sites and on fewer host *Chionochloa* species (Kelly *et al*., 2000).

If the cecidomyiid is a single insect species attacking all species of *Chionochloa* across a wide geographic range and with predictive diapause, it would be expected to impose strong selective pressure on *Chionochloa* spp. to flower in synchrony (among species and regions) with a high level of variation. *Chionochloa* species flower in synchrony both among species and across wide geographic ranges (Kelly *et al*., 2000; Schauber *et al*., 2002), consistent with selection pressure from a single widespread species of seed predator. Unfortunately, much of the natural history of the cecidomyiid is unknown, since the adults are extremely difficult to rear under laboratory conditions and have only been collected on two occasions in the field, with most of those caught being female (see drawings of larvae and adults in McKone *et al*., 2001). Thus, it is not possible to determine, based on morphology alone, whether there is a single species of cecidomyiid present on many species of *Chionochloa* or specialised races or species of cecidomyiid on different species of *Chionochloa*. Specialisation based on host plant species is possible, given the wide radiation of *Chionochloa* into diverse habitats (Edgar and Connor, 2000) and the existence of specialised insect host races in other systems (Bush, 1994; Brown *et al*., 1996). It is also possible there are different species or races of the cecidomyiid present at different locations within New Zealand, produced by allopatric processes.

If each *Chionochloa* species has a specialised seed predator, flowering synchrony among species could simply be due to different *Chionochloa* species using the same abiotic clue to synchronise flowering (Norton and Kelly, 1988; Kelly, 1994; Kelly *et al*., 2000).

Given the uncertain taxonomic status of the undescribed cecidomyiid and its possible importance in the maintenance of *Chionochloa* masting, this study addresses two main questions: 1) Do the undescribed cecidomyiid larvae that have been observed on many *Chionochloa* hosts and in many areas in New Zealand belong to the same species?, and 2) If the cecidomyiid larvae belong to the same species, how much quantifiable genetic differentiation is there among and within host plant populations and geographic regions?

Since insufficient numbers of cecidomyiid adults were available for identification based on morphological features, we used the molecular method of Inter Simple Sequence Repeats (ISSRs) to determine the putative conspecific status among cecidomyiid larvae on different host species of *Chionochloa* and in different geographic regions.

Materials and methods

Inter Simple Sequence Repeats (ISSRs)

Molecular characteristics of individual cecidomyiid larvae were determined using 5'- anchored Inter Simple Sequence Repeat (ISSR) primers. These dominant markers amplify highly repetitive short sequences in DNA and are common in the eukaryotic genome. ISSR primers are designed to amplify repetitive DNA sequences like Simple Sequence Repeats (SSRs) and thus have microsatellite sequences in the primer, but also contain random DNA sequences, similar to Random Amplified Polymorphic DNA (RAPD) primers (Zietkiewicz *et al*., 1994). Despite the random

nature of the primers, ISSRs appear to be more stable than RAPDs because they have longer primer sequences and use a higher annealing temperature in the Polymerase Chain Reaction (PCR) protocols (Wolfe *et al*., 1998; Nagaraju *et al*., 2001). ISSR markers have been used extensively to determine plant population differentiation (Fang *et al*., 1998; Wolfe *et al*., 1998; Chapman *et al*., 2000) and to determine insect population differentiation in the silkworm *Bombyx mori* (Reddy *et al*., 1999; Nagaraju *et al*., 2001), the parasitic wasp *Microtonus aethiopoides* (Phillips *et al*., 2002) and in a parasitic Tachinid fly (Chatterjee *et al*., 2003).

Nagaraju *et al*. (2001) compared four types of molecular marker [Restriction Fragment Length Polymorphisms (RFLPs), RAPDs, ISSRs and SSRs] in their ability to differentiate among 13 silkworm (*Bombyx mori*) varieties. A quantitative index of marker diversity, the Marker Index (MI), was used to identify the relative efficiency among the different types of markers. The formula for MI is complex, but, in short, it is proportional to the product of the mean heterozygosity of each marker and the proportion of polymorphic loci for that marker (Nagaraju *et al.* 2001). All markers successfully differentiated the different silkworm varieties, but the ISSRs and RFLPs were the most efficient, with MIs of 22.57 and 26.67 respectively (MIs for SSRs = 0.80 and RAPDs = 3.58). Thus, ISSRs appear to have some utility in distinguishing insect populations and have a relatively high yield of information compared with other markers.

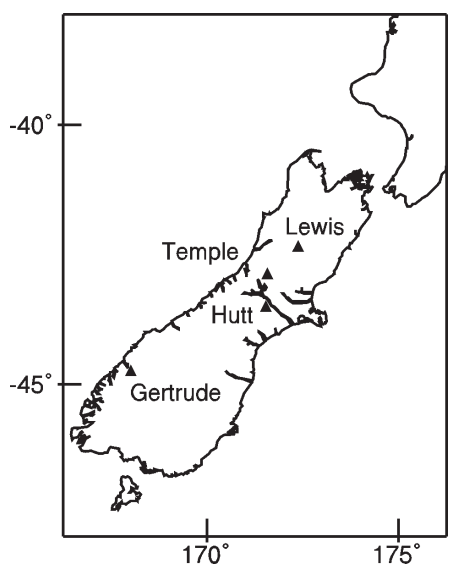

Figure 1. Map of the South Island of New Zealand showing the four study locations where cecidomyiid larvae were collected for ISSR analyses. Gertrude = Gertrude Saddle, Hutt = Mt. Hutt, Lewis = Lewis Pass, Temple = Temple Basin.

DNA extraction and amplification

A total of 82 undescribed cecidomyiid larvae from four different *Chionochloa* geographic sites within the South Island of New Zealand were collected for molecular marker analysis (Table 1, Fig. 1). The sites were chosen so two or more species of *Chionochloa* were present in each site (Table 2) to determine the relative effects of host plant and geography on cecidomyiid genetic differentiation. In total, insects from four different species of *Chionochloa* were collected: *C. crassiuscula*, *C. oreophila*, *C. pallens* and *C. rubra*. The altitude and landscape varied within each site (Table 1), but each host-plant population appeared to be continuous. Inflorescences from individual *Chionochloa* plants were haphazardly selected at each site and were either desiccated using silica gel or placed on ice at 4° C until dissections were made. At all stages of the collection phase, inflorescences from individual plants were kept separate from other plants. Individual cecidomyiid larvae were then dissected from the *Chionochloa* florets and were frozen at –80° C until we conducted the molecular analyses.

In addition, one gall-making species of cecidomyiid (R. MacFarlane, *pers. comm.*, Canterbury Museum, Christchurch, N.Z.) was collected from a species of *Hebe* at the Mt. Hutt study site in order to serve as an outgroup for the clustering analysis. We were unable, however, to identify this cecidomyiid to species level based on larval morphology alone, although it is probably in the same subfamily, Cecidomyiinae, as the *Chionochloa* cecidomyiid (R. Macfarlane, *pers. comm.*). Ideally, the variation among ISSR marker frequencies would be high enough

Table 1. Descriptions and elevations of the four sites used in this study.

Site	Latitude S/ and Longitude E	Elevation Range	Description
Gertrude Saddle	44° 44´ S 168° 01´ E	1030–1100 m	Undisturbed saddle
Lewis Pass	42° 23´ S 172° 23´ E	860–1260 m	Parking lot (*C. rubra*), undisturbed saddle
Mt. Hutt	43° 32´ S 171° 32´ E	450–1070 m	Roadside (*C. rubra*), skifield
Temple Basin	42° 55´ S 171° 35´ E	890–1350 m	Parking lot (*C. rubra*), skifield

Table 2. Summary of insects collected and analyzed using ISSR primers. One cecidomyiid was collected from a *Hebe* sp. at Mt. Hutt to use as an outgroup, yielding a total of 83 insects.

Site	*C. crassiuscula*	*C. oreophila*	*C. pallens*	*C. rubra*	Totals
Gertrude Saddle	6	0	16	0	22
Lewis Pass	13	2	0	4	19
Mt. Hutt	0	0	8	8	16
Temple Basin	8	0	11	6	25
Totals	27	2	35	18	82

to discriminate between the *Chionochloa* and *Hebe* cecidomyiids, which are presumably separate species.

Several recent studies have used ISSRs to successfully identify different geographic populations of insects (Reddy *et al*., 1999; Nagaraju *et al*., 2001; Phillips *et al*., 2002; Chatterjee *et al*., 2003), so ISSRs could likewise have the ability to distinguish populations within the *Chionochloa* cecidomyiid. If we find that ISSRs can distinguish between species (like the *Hebe* and *Chionochloa* cecidomyiids) and we know that ISSRs can distinguish populations within insect species, then it is probable that ISSRs have enough variation to distinguish between reproductively-isolated, but closely-related species of the *Chionochloa* cecidomyiid if they exist.

Cecidomyiid DNA was extracted using the Wizard Genomic Purification Kit (ProMega) and amplified in 25-µL reactions containing 1.25 units *Taq* polymerase (Boehringer-Mannheim), 2.5 µL 10x PCR buffer (Boehringer-Mannheim), 3 µM $MgCl_2$, 0.5 µM DNTPs, 0.4 µM ISSR primer (University of British Columbia), and approximately 40 ng of DNA template. Each reaction was overlaid with 10 µL of liquid paraffin to ensure uniform heating of samples and preparation of samples was performed in a laminar flow hood to prevent contamination. Samples were incubated in a Peltier thermocycler (Model PTC-200, MJ Research, Inc.) using the following reaction profile: 4 min at 93° C followed by 40 cycles of the following sequence: 20 s at 93° C, 60 s at 52° C, and 20 s at 72° C. The final step was a 4 min extension at 72° C. Samples were run on 2.0% agarose gels containing ethidium bromide for visualisation. Every fourth sample was run a second time to ensure the reproducibility of bands; samples yielding indistinct banding patterns were re-run to determine pattern consistency and bands that were not consistent among runs were not scored.

A total of 16 different ISSR primers were initially screened for reproducible, intermediate-weight DNA fragments. Seven primers (Table 3) were chosen to analyse the 83 insects in the study. We scored 38 bands for each of the cecidomyiid larvae in the study using a presence/absence criterion with the assumption that each marker represented a single locus.

Table 3. ISSR primers (University of British Columbia primer set #9) used in this study. R refers to a random purine base, Y refers to a random pyrimidine base.

UBC Primer Number	Sequence 5'—3'
842	GAGAGAGAGAGAGAGAYG
845	CTCTCTCTCTCTCTCTRG
848	CACACACACACACACACARG
853	TCTCTCTCTCTCTCTCRT
867	GGCGGCGGCGGCGGCGGC
873	GACAGACAGACAGACA
899	CATGGTGTTGGTCATTGTTCCA

Genetic distances and cluster analysis

A pairwise distance matrix among all possible binary combinations of samples was computed using Nei and Li's genetic distance metric (Nei and Li , 1979) with the program MVSP 3.0 (Multi-Variate Statistical Package, Kovach Computing Services, Wales, U.K.). Nei and Li's genetic distance is:

$D_{ij} = 1\text{-}(2a/(2a + b + c))$,

where a is the number of times samples i and j match for the presence of a band and b and c are instances where there is a mismatch between samples. Genetic distance was also computed within and between geographic regions and within and between host-plant populations using MVSP 3.0. Molecular gene diversity over all loci was also computed for the geographic regions and host-plant populations using the program Arlequin (V 2.00, S. Schneider, D. Roessli, L. Excoffier, University of Geneva). Dendrograms employing the Nei and Li distance matrix for the cecidomyiid larvae were constructed using the UPGMA clustering method in PAUP* [Phylogenetic Analysis Using Parsimony (* and other methods), Version 4.0, Swofford, D.L. 2002. Sinauer Associates, Sunderland, Massachusetts, U.S.A.].

Analysis of Molecular Variance (AMOVA)

Variance among and within host-plant and geographic

populations was analysed using the AMOVA (Analysis of Molecular Variance) technique developed by L. Excoffier and colleagues (Excoffier *et al*., 1992). Although this technique was originally designed for use with restriction fragment data, the AMOVA process is routinely used to analyse population subdivision using dominant markers such as RAPDs (Huff *et al*., 1998; Bartish *et al*., 1999). We conducted a nested AMOVA (e.g. Russell *et al*., 1999) with host-plant populations nested within the geographic regions in order to determine the relative contribution of host plant and geography to the overall molecular variance. We also ran separate single-category AMOVAs to determine the amount of genetic differentiation among the geographic regions and the host-plant populations by themselves. The AMOVA analyses were run using the Arelquin program (V 2.00, S. Schneider, D. Roessli, L. Excoffier, University of Geneva).

The main measure of population differentiation in AMOVA is the ϕ statistic, which is homologous to the *F*-statistics described by Wright (see Excoffier *et al*., 1992). It is important to note that this analysis is basically phenetic in character, since actual heterozygote frequencies at each marker "locus" are unknown.

Significance testing in AMOVA is based on permutation analysis, where artificial populations are created from random samples drawn from the observed population. The amount of differentiation among the artificial populations is then compared with the differentiation values for the total actual populations; the probability of the artificial populations resulting in an equal or higher amount of differentiation than the actual populations is then computed by repeating the comparison 1000 times. If the ϕ statistic for the actual populations is exceeded by the random populations in less than 50 cases ($P = 0.05$), then the null hypothesis of no significant population differentiation among the original populations can be rejected (Excoffier *et al*., 1992).

Results

Out of the 38 markers scored in the ISSR analysis, 36 were polymorphic among the *Chionochloa* cecidomyiids. The percentage of unique genotypes across geographic populations varied from 84% at Temple Basin to only 32% at Gertrude Saddle (Table 4). The low number of unique genotypes found at Gertrude Saddle corresponds to the low amount of molecular variation found among all samples at that site, as determined by the AMOVA analysis (Table 4). Twenty larvae originating from all sites had identical ISSR banding patterns (Fig. 2), but were only found on *Chionochloa pallens* and *C. rubra*.

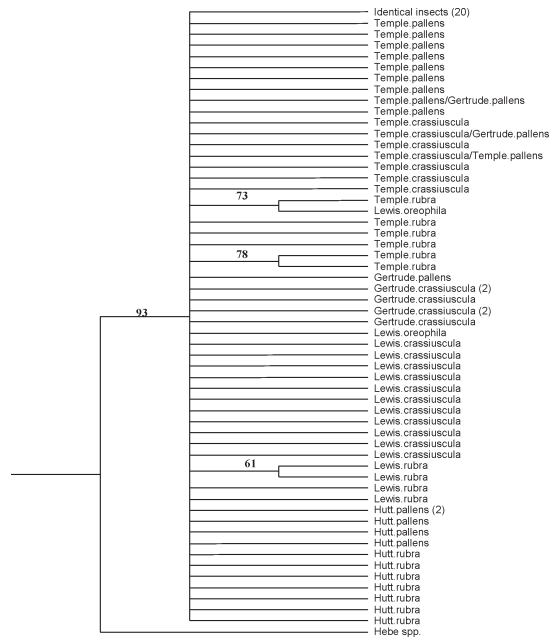

Figure 2. UPGMA dendrogram with 1000 bootstrap replications showing the relationships among the cecidomyiid larvae from *Chionochloa* and *Hebe* populations. The first term designates the collection site and the second term designates the *Chionochloa* host plant species. Only bifurcations present in greater than 50% of the bootstrap replicates are reported. Numbers in parentheses indicate how many identical genotypes are present at a particular branch tip. The branch tip containing 20 identical, "clonal" insects is comprised of insects from a variety of collection sites and host plants.

Table 4. Percentage of unique genotypes and the molecular variance within each study site. Molecular gene diversity over all 38 loci was determined using the program Arlequin (V. 2.00).

Site	# Unique genotypes	# Total samples	% Unique genotypes	Molecular gene diversity
Gertrude Saddle	7	22	32	0.034
Lewis Pass	16	19	84	0.239
Mt. Hutt	12	16	75	0.179
Temple Basin	18	25	72	0.227

The dendrogram using the UPGMA clustering technique with bootstrap support showed no clear groups of cecidomyiids based on either host-plant population or geographic region (Fig. 2). Similarly, there were no clear groups within each geographic region based on altitude (data not shown). The cecidomyiid from the *Hebe*, however, was grouped separately from the *Chionochloa* cecidomyiids with high bootstrap support, suggesting that the 38 ISSR markers are sufficient to differentiate between two separate species of cecidomyiid, although the exact phylogenetic relationship between the *Chionochloa* and *Hebe* cecidomyiids is unknown. This result, along with the results from Nagaraju *et al*. (2001) and Chatterjee *et al*. (2003) suggest that ISSRs can be useful in determining reproductively-isolated species and populations within species.

Single-category AMOVAs demonstrated significant variation attributable to geographic regions (15.9%) and host-plant population (10.0%), although most of the variation was due to within-region and within-host population variation (Table 5). The nested AMOVA analysis showed that most of the variation among individual cecidomyiid larvae was found within host-plant populations (Table 6). The amount of variation attributed to either geographic region (9.4%) or host -plant population (14.1%) was small compared with the within host-plant variation (76.6%), although the *F* statistics for all hierarchical levels were significant at the 0.05 level.

The mean genetic distance was 0.108 between geographic regions and 0.124 within regions. The mean genetic distance was 0.160 among host-plant populations and 0.161 within host populations (Table 7).

Table 5. Single category AMOVAs based on geographic region and host plant species. ϕ values are indices of the amount of differentiation among populations, similar to Wright's *F* statistics. All analyses were performed without the *Hebe* cecidomyiid. d.f. = degrees of freedom, SS = sum of squares.

Source of Variation	d.f.	SS	Variance components	% of variation	ϕ	*P*
Among geographic regions	3	46.661	0.607	15.90	0.159	<0.001
Within geographic regions	78	250.595	3.213	84.10		
Totals	81	297.256	3.820			
Among host species	3	30.955	0.382	10.04	0.100	<0.001
Within host species	78	266.301	3.414	89.96		
Totals	81	297.256	3.796			

Table 6. Summary of a nested AMOVA with host-plant species nested within geographic regions. ϕ values are indices of the amount of differentiation among populations, similar to Wright's *F* statistics. All analyses were performed without the outgroup *Hebe* cecidomyiid. d.f. = degrees of freedom, SS = sum of squares.

Source of Variation	d.f.	SS	Variance components	% of variation	ϕ	*P*
Among geographic populations	3	46.66	0.358	9.36	0.094	0.029
Among host species within geographic populations	6	40.09	0.538	14.08	0.155	0.003
Within host species	72	210.50	2.927	76.56		
Totals	81	297.25	3.823			

Table 7. Survey of insect population differentiation studies using dominant markers. All studies employed RAPDs except for this study, which used ISSRs. All studies used Nei and Li's (1979) distance measure and were performed on individual species. Differentiation was determined by the authors of each study.

Species	Genetic distance	Genetic distance between/ genetic distance within	Differentiation	Reference
Bactrocera cucurbitae (Diptera: Tephritidae)	0.905 between populations, 0.474 within	1.91	Yes	Haymer (1995)
Ceratitis capitata (Diptera: Tephritidae)	0.523 among populations, 0.443 within	1.18	No	Haymer *et al.* (1997)
Listronotus bonariensis (Coleoptera: Curculionidae)	0.672 between populations, 0.555 within populations	1.21	Yes	Williams *et al.* (1994)
Ostrinia nubilalis (Lepidoptera: Pyralidae)	0.344 among biotypes, 0.089 within	3.87	Yes	Pornkulwat *et al.* (1998)
Plodia interpuntella (Lepidoptera: Pyralidae)	0.291 among sites, 0.096 within	3.03	Yes	Dowdy and McGaughey (1996)
Chionochloa cecidomyiid	0.160 among hosts, 0.161 within	0.99	No	This study
Chionochloa cecidomyiid	0.108 among sites, 0.124 within	0.87	No	This study

Discussion

The results from the cluster analysis suggest that there is little genetic differentiation among the cecidomyiid larvae based on host-plant population, geographic site or altitude. Although there were statistically significant differences among insect populations based on geography and host plant using the AMOVA technique, we believe this differentiation is not indicative of significant biological differences among populations. For example, although there were statistically significant differences among geographic regions and among nested host species in the nested AMOVA, a far greater amount of the variance among samples was explained *within* host populations. Similarly, single-category AMOVAs showed significant differentiation among geographic regions and host-plant populations, but most of the total variation was found within geographic regions and host-plant populations. Furthermore, the magnitude of the ϕ values in the AMOVA among geographic regions and host populations were comparable to ϕ values reported among populations of grape phylloxera, which were determined to be biologically undifferentiated despite the significant statistical differentiation among populations (Downie, 2000).

The genetic distances within and among geographic regions and host-plant populations also suggest a clear lack of differentiation based on these groupings. If the cecidomyiids were strongly differentiated according to geographic site or host plant, then the genetic distance among these populations would presumably be larger than the genetic distance within populations. A short review of insect studies employing both dominant markers and Nei and Li's genetic distance shows that significant population differentiation (subjectively determined by the authors) is usually accompanied by large between-population distance to within-population distance ratios (Table 7). In cases where differentiation has occurred, the ratio of between-population to within-population genetic distance is greater than one, but these ratios are less than one for both the geographic populations and host-plant populations in the undescribed cecidomyiid.

The absence of differentiation among both geographic and host-plant populations in the

cecidomyiids may have been caused by at least three mechanisms. First, panmixis of genotypes could occur through the wide dispersal of cecidomyiids throughout the South Island. The cecidomyiids are capable of flight and may be aided in dispersal by high winds that have been shown to have an impact on the flight patterns of another member of the Cecidomyiidae, *Mayetiola destructor* (Withers and Harris, 1997).

Second, it is possible that the current range of the *Chionochloa* cecidomyiid is not coincident with the ancestral range. Specifically, a small ancestral cecidomyiid population could have undergone panmixis in refugia during the Pleistocene, followed by a rapid range expansion after the retreat of glaciers. Lack of molecular variation has often been attributed to similar recent colonisation events (Reiss *et al*., 1999). Alternatively, McGlone (2001) suggested that both low-altitude and some inland alpine *Chionochloa* populations in the South Island expanded quickly following the burning of woody areas by the first human colonists of New Zealand. Either of these possibilities, if the range expansion was rapid and recent enough, could result in a relatively well-mixed and thus undifferentiated present distribution of cecidomyiid genotypes.

Finally, host-plant phenology may contribute to the observed lack of genetic differentiation. All of the *Chionochloa* species we examined flower at approximately the same time in the summer and have similar-sized seeds, and our study species are largely widespread across the South Island. If insects from different plants mate on or near their host plant, then interbreeding among insects from different hosts or adjacent regions could homogenize the genetic variation in the insect species.

Curiously, the population at Gertrude Saddle was relatively monomorphic for the 38 ISSR markers used in this study, as shown by the low molecular gene diversity at the site (Table 4). This uniformity may be explained by several mechanisms. First, simple sampling error in the field could have biased the larvae collection in such a way that all the sample insects belonged to closely-related female flies. It is difficult to dismiss this explanation without further sampling at Gertrude Saddle, although sampling was conducted in a similar manner at all sites. Alternatively, the population at Gertrude Saddle may have arisen through the establishment of only a few individuals from other areas. Given that the most common genotype at Gertrude Saddle is also found at the other sample sites and that Gertrude Saddle is encircled by the relatively high Darren Mountain range, this "founder effect" is possible. Finally, the population at Gertrude Saddle may exhibit an unusual reproductive system that produces parthenogenic larvae. Within the family Cecidomyiidae there are certain groups that are known to reproduce through parthenogenic means (Wyatt 1961; 1967), so this situation is not unprecedented. Despite this possibility, we believe that parthenogenesis is unlikely since males and females of the adult cecidomyiid have now been found in the field at the Mt. Hutt site (*unpub. data*).

Given the lack of strong differentiation among geographic regions and host-plant populations, it appears likely that the cecidomyiids found in the four collection sites and on different *Chionochloa* host plants belong to the same species. How does this conclusion affect the predator satiation hypothesis? Since many *Chionochloa* species in different geographic areas mast in synchrony (Kelly *et al*., 2000; Schauber *et al.*, 2002), synchrony may be adaptive if all the plant species are responding to the same seed predator. This study confirms conclusions based on the gross morphology of larvae found in prior *Chionochloa* seed predator surveys; the cecidomyiid is the most general and widespread of the major seed predators found on *Chionochloa* species in New Zealand (McKone *et al*., 2001). Given the large losses due to cecidomyiid seed predation in *Chionochloa* species (McKone *et al*., 2001), our results suggest that the undescribed cecidomyiid may be an important agent in the maintenance of inter-specific masting in *Chionochloa*.

Two remaining questions are relevant to evaluating the selective pressure on masting in *Chionochloa* posed by the cecidomyiid. First, the cecidomyiid is thought to be specific to the genus *Chionochloa* (McKone *et al*., 2001), but there have been few systematic searches for it in other plant genera. The degree of host specialisiation of the insect determines how vulnerable it is to starvation in years of low flowering by *Chionochloa*. Second, and related to the above, is the possibility of extended diapause in the cecidomyiid, which would allow it to wait through non-flowering years, then emerge in subsequent flowering years (Kelly *et al*., 2000). It would be worthwhile to verify experimentally whether extended diapause is present in this insect, and what cues trigger emergence.

In conclusion, the undescribed cecidomyiid larvae found at four widely-separated sites throughout the South Island of New Zealand and on different *Chionochloa* host plants are likely to belong to the same species of insect and are relatively undifferentiated across a large geographic range. This finding supports the hypothesis that the undescribed cecidomyiid is general in its preferences for host plant and habitat and may be partially responsible for the across-species and across-site synchrony in masting observed in many South Island *Chionochloa* species.

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