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# Genetic diversity of introduced populations of the water hyacinth biological control agent *Eccritotarsus catarinensis* (Hemiptera: Miridae)

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ABSTRACT

Genetic bottlenecks can be deleterious to populations. In biological control, agent populations may be subject to severe bottlenecks during selection, importation and while in culture. The genetic variability of two collections of the water hyacinth biological control agent *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) was measured using Inter-simple Sequence Repeats (ISSR) and mtDNA cytochrome oxidase I (COI) sequences. The first collection (Brazilian) went through a bottleneck of a single gravid female, while the second collection (Peru) originated from 1000 individuals and has been maintained at a large size in culture. Two naturalised South African populations from the Brazilian collection were also sampled (Nseleni and Mbozambo). Polymorphism for ISSR was high in the Peruvian and two naturalised samples, but much less so in the Brazilian sample. The Peruvian population was shown to be highly differentiated from the Brazilian and its naturalised populations by high values of $F_{ST}$ and Nei's genetic distance, as well as in a Multidimensional Scaling (MDS) plot and an unrooted neighbour joining tree derived from Jaccard's coefficient of similarity. In addition, sequencing of the COI region of the mitochondrial DNA revealed only two haplotypes, one Brazilian and one Peruvian, with a 5.2% sequence divergence, suggesting that recombination and not mutation is the cause of most variation in the ISSR regions. The results suggest that substantial genetic variation may be retained or recovered after a bottleneck. This may mitigate deleterious effects that are a concern for the fate of biological control agents after release.



## 1. Introduction

In biological control, founder population effects and genetic bottlenecks have not often been researched (but see Grevstad, 1999; Baker et al., 2003; Hufbauer et al., 2004; Franks et al., 2010). However, the nature of biological control is such that genetic bottlenecks could be important and need to be considered. In the process of selecting a biological control agent, a subset of the original population is sampled, reared in quarantine for host specificity testing and subsequently released. Founder effects could be deleterious and have repercussions in the future of these populations (Griffen and Drake, 2008). In addition to the initial bottleneck that agent populations go through, insects are kept in quarantine for up to 12 years while release evaluations are conducted and permission for release is granted (Bownes et al., 2010). This can cause population suppression resulting in multiple bottlenecks, potentially impacting population diversity (Leberg and Firmin, 2008). However, once insects are released and naturalised, abundant resources allow for a population explosion which may increase diversity (Nei et al., 1975).

Frankham (2005) presented compelling evidence that genetic bottlenecks have a deleterious effect on fitness in populations and play a role in extinction. This has been demonstrated many times in laboratory studies (Radwan, 2003; Briskie and Mackintosh, 2004; Leberg and Firmin, 2008) and should not be ignored when considering the fate of small populations. However, in some cases, small population size and lowered genetic diversity may play a role in purging populations of deleterious alleles, leading to increased population fitness (Jamieson et al., 2006). Despite this, bottleneck size has been shown to be negatively proportional to fitness (Briskie and Mackintosh, 2004), and purging has been found experimentally to cause lower fitness and higher extinction rates in laboratory experiments (Radwan, 2003; Leberg and Firmin, 2008).

Nei et al. (1975) showed that loss of heterozygosity in populations that have been subjected to genetic bottlenecks is determined not only by the size of the bottleneck, but also by the rate of population increase. Rapid population expansion can restore or prevent the loss of substantial genetic variation, and increase the chance that only rare alleles will be lost during the bottleneck, leaving variation less affected (Nei et al., 1975; Lande, 1980; Slatkin, 1985). However, loss of alleles due to a bottleneck can reduce the ability of the population to survive environmental

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stochasticity and constrain adaptation to new environments (Frankham, 2005).

Long-term fluctuation of numbers through population contraction and expansion can cause multiple bottlenecks which reduce variation within populations even further than a single bottleneck event. The average effective population size ($N_E$) is influenced most strongly by periods of contraction (Hartl and Clark, 2007). If a population undergoes multiple bottlenecks then there is a lower likelihood of that population surviving (Leberg and Firmin, 2008) but population explosion and available resources can increase chances of survival of released populations (Schoener and Schoener, 1983).

The mirid bug, *Eccritotarsus catarinensis* Carvalho, was collected in 1992 from Florianopolis, Brazil (Hill et al., 1999), as a biological control agent of invasive water hyacinth (*Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae)). While in quarantine in South Africa, the majority of the culture died and only one gravid female survived to found the current *E. catarinensis* population in South Africa (Hill, pers. comm.). However, after its release in 1996 the population has expanded rapidly and is established at more than 30 sites around South Africa forming multiple sub-populations (Fig. 1) (D. Schlange, unpubl.). *E. catarinensis* and other biological control agents of water hyacinth have been highly successful in sub-Saharan Africa, but their success seems to have been limited in South Africa by the variability of the South African climate, eutrophication of water systems and interference from other control options (Hill and Olckers, 2001).

*E. catarinensis* particularly, is limited by colder temperatures; over winter the populations die back with the water hyacinth, but in the summer the water hyacinth has the ability to regenerate faster than the mirid populations (Coetzee et al., 2007). In light of this, a new population of the mirid was sampled from the upper Amazonian basin, Yarapa River, Peru in 1999 (Hill, pers. comm.). This region has an altitude of about 650 m above sea level making this probably the highest elevation population of *E. catarinensis* in the world. Yarapa River has a colder climate than Florianopolis, Brazil, and so mirids collected here could be better adapted to lower temperature ranges satisfying the ideal of climate matching to the introduced region (Williamson, 1996). Despite predictions, preliminary laboratory studies have suggested that the Peruvian collection does not have a lower temperature tolerance than the Brazilian collection (Voogt, unpubl.), nor do they differ in feeding damage (Price, unpubl.).

A collection of more than 1000 individuals was made from Peru and this culture has not gone through a similar bottleneck to the Brazilian population (Hill, pers. comm.). Therefore the inclusion of individuals from this population into the South African population should serve to broaden the genetic variation of *E. catarinensis* in South Africa. The Peruvian collection has been in culture for almost 10 years and has not mixed with naturalised Brazilian insects due to its very recent release at sites where the mirid was absent. The population size in culture is not known for either of the two populations but numbers do fluctuate seasonally (Coetzee, pers. comm.).

Levels of genetic variation are likely to have important implications for the process of selecting, quarantining and introducing biological control agents, and the successes and failures of these insects. The aims of this study were to explore the hypothesis that the genetic variation of the Peruvian collection of *E. catarinensis* would be greater than that of the Brazilian collection due to a greater founder population size, and to quantify the genetic diversity within and divergence among the collections of the mirid from both source populations and naturalised populations of the Brazilian collection to assess the genetic consequences of the bottleneck.

## 2. Materials and methods

### 2.1. Sampling

Four populations were sampled, two in culture and two naturalised. The Peruvian collection of *E. catarinensis* is currently in quarantine at Rhodes University, Grahamstown, South Africa and 10 individuals were sampled from this population ($P_1 - P_{10}$). Ten indi-

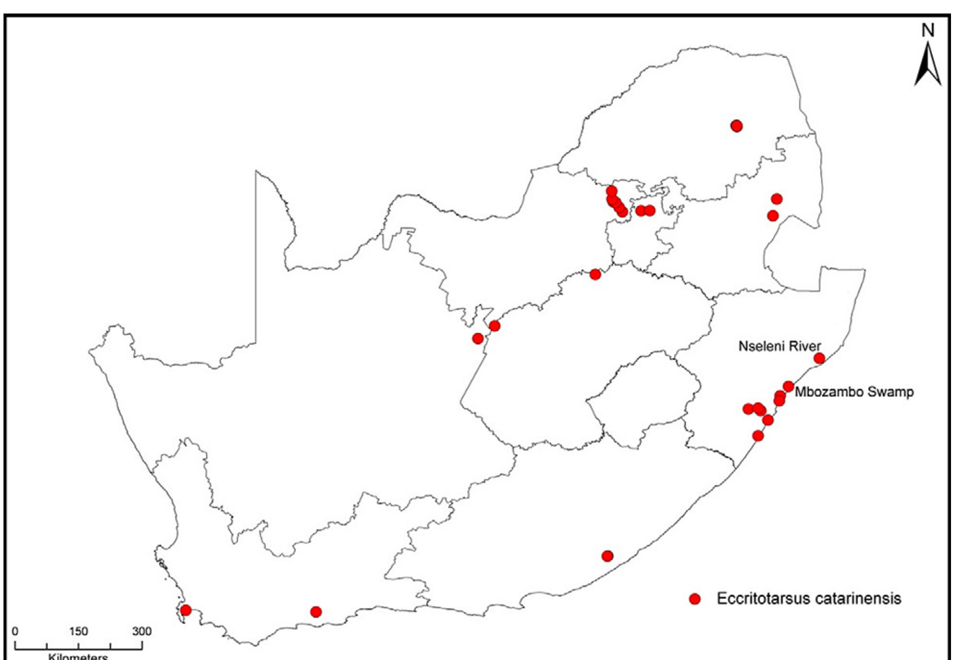


**Fig. 1.** Sites of establishment of Brazilian *Eccritotarsus catarinensis* as a biological control agent on water hyacinth in South Africa (D. Schlange, unpubl.). The two sites of naturalised *E. catarinensis* populations that were sampled for this study are labelled.