Unravelling the Paradox of Loss of Genetic Variation during Invasion: Superclones May Explain the Success of a Clonal Invader

Valerie Caron¹*, Fiona J. Ede², Paul Sunnucks¹

¹School of Biological Sciences, Monash University, Clayton, Victoria, Australia, ²Biosciences Research Division, Department of Environment and Primary Industries, Bundoora, Victoria, Australia

Abstract

Clonality is a common characteristic of successful invasive species, but general principles underpinning the success of clonal invaders are not established. A number of mechanisms could contribute to invasion success including clones with broad tolerances and preferences, specialist clones and adaptation in situ. The majority of studies to date have been of plants and some invertebrate parthenogens, particularly aphids, and have not necessarily caught invasion at very early stages. Here we describe the early stages of an invasion by a Northern Hemisphere Hymenopteran model in three different land masses in the Southern Hemisphere. Nematus oligospilus Förster (Hymenoptera: Tenthredinidae), a sawfly feeding on willows (Salix spp.), was recently introduced to the Southern Hemisphere where it has become invasive and is strictly parthenogenetic. In this study, the number of N. oligospilus clones, their distribution in the landscape and on different willow hosts in South Africa, New Zealand and Australia were assessed using 25 microsatellite markers. Evidence is presented for the presence of two very common and widespread multilocus genotypes (MLGs) or ‘superclones’ dominating in the three countries. Rarer MLGs were closely related to the most widespread superclone; it is plausible that all N. oligospilus individuals were derived from a single clone. A few initial introductions to Australia and New Zealand seemed to have occurred. Our results point towards a separate introduction in Western Australia, potentially from South Africa. Rarer clones that were dominant locally putatively arose in situ, and might be locally favoured, or simply have not yet had time to spread. Data presented represent rare baseline data early in the invasion process for insights into the mechanisms that underlie the success of a global invader, and develop Nematus oligospilus as a valuable model to understand invasion genetics of clonal pests.

Citation: Caron V, Ede FJ, Sunnucks P (2014) Unravelling the Paradox of Loss of Genetic Variation during Invasion: Superclones May Explain the Success of a Clonal Invader. PLoS ONE 9(6): e97744. doi:10.1371/journal.pone.0097744

Editor: Youjun Zhang, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Science, China

Received December 12, 2013; Accepted April 24, 2014; Published June 10, 2014

Copyright: © 2014 Caron et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This research was funded by Holsworth Endowment, the Department of Primary Industries Nancy Mills Postgraduate Award, North East Catchment Management Agency, West Gippsland Catchment Management Agency, North Central Catchment Management Agency, Melbourne Water and the Victorian Department of Primary Industries Nancy Mills Postgraduate Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: valerie.caron@monash.edu

Introduction

One of the major paradoxes of invasion biology is how species with very low genetic variation (and thus with expected low evolutionary potential) can still be successful invaders [1,2,3,4]. As invaders usually arrive in very low numbers, genetic bottlenecks should reduce genetic diversity in the invasive range; for example: [5,6,7]. A number of hypotheses have been suggested to resolve this paradox. The first is that that genetic/genomic diversity is often not lost, but when it is, phenotypic variation may not be reduced due to plasticity [8]. In some cases, genetic diversity in the invasive range may increase owing to admixture between individuals from multiple sources [1,9,10]. Alternatively, it has been proposed that rapid evolution generates functional diversity and/or local adaptation [4,11].

One commonly observed feature of invasive species is the tendency to become clonal in the invasive range, which has been suggested to enable invasive organisms to thrive in the face of low diversity [2]. Clonal organisms are more than four times over-represented among pest invertebrates [12]. Clonality has been highlighted as an important mechanism by which invaders with low genetic diversity succeed, and a meta-analysis uncovered the pattern that a disproportionate fraction of aquatic invaders with low diversity were asexual [2]. In addition to the demographic benefits of asexuality (notably not investing in males, and that a single individual can found a new colony), this mode of reproduction is generally impervious to inbreeding depression and fit genotypes are not broken up by sexual recombination [2]. Conversely, while lack of the ability to shuffle pools of alleles into new genotypic combinations is on face value disadvantageous, ecological traits of parthenogens with large population sizes nonetheless can diversify quickly by mutation, and rapid evolution is an increasingly recognized contributor to invasiveness [13,14].

It is common for invasive organisms to transition to obligate or functional parthenogenesis; for example: [15,16,17,18]. These transitions result in the unit of selection switching instantaneously from the gene to a co-inherited suite of genomes (nuclear, mitochondrial, symbionts) at least when the transitions are irreversible [19]. This has important implications for the diversity of genotypes and phenotypes, and the fitness consequence of their
interactions with environmental conditions, and thus for control strategies [12]. An invasion that produces clones might be characterized by a large or small number of fixed clones, or some complex scenario of asexual/sexual interactions and recurrent generation of new clones. Natural selection is generally expected to act rapidly among clonal lineages, particularly when the number of individuals within lineages is large, the number of lineages small, and they persist over time [20]. Pre-adapted genotypes should be favoured, while strong selection against suboptimal genotypes should occur soon after introduction. Genotypes may be thought of as more or less ‘generalist’ or ‘specialist’ based on the breadth of their ecological niche and their environmental tolerances which can translate into their distributions in environmental space and time; both can lead to successful invasion [16,21,22,23]. New phenotypes can arise following mutations and karyotypic changes [4,11,24] and it is of central interest in invasion genetics to what extent this is an effective part of invasion ability of clonal organisms.

A high proportion of studies on these topics have been of cyclic parthenogens; in particular, Hemiptera, and more specifically aphids, are extremely well-represented in pest species and studies of them [12,25]. This important area of study requires strong models encompassing diverse organisms, with longitudinal studies starting early in invasions, ideally in multiple locations, applying highly-resolving genetic assays. A promising emerging model for understanding invasions of clonal organisms with low diversity is the willow sawfly Nematus oligospilus Forster (Hymenoptera: Tenthredinidae). This taxon has recently invaded the Southern Hemisphere [26]. It is native to Eurasia (from Ireland to the Himalayas) [27] and North America (from Alaska to Mexico) [28]. However, recent research points towards N. oligospilus representing a group of species and the native range of the invasive species is unknown [26,29]. It was first detected in South America in 1980 [30,31]. By 1993 it had spread to southern Africa [32], it reached New Zealand by 1997 [33] and was first detected in Australia (Canberra, Australian Capital Territory) in 2004 [34]. By 2009, it had established in most of southeast Australia (from South Australia to the New South Wales and Queensland border), Tasmania and southwestern Western Australia and was still undergoing expansion [35].

A recent study developed a high-resolution microsatellite assay and identified several multi-locus genotypes (MLGs) in N. oligospilus in its invasive range in the Southern Hemisphere [26]. In this paper, we seek to advance the understanding of the evolution of invasive parthenogens that diverge without sexual reproduction and genetic recombination. In addition, we identify N. oligospilus as an exciting model taxon of parthenogenetic invasion. Here we assess the distribution, genetic relatedness and host associations of N. oligospilus MLGs in South Africa, New Zealand and Australia from widespread, substantial sampling very early in the invasion process. Specifically, we address the following questions: 1/What clones of N. oligospilus are detectable in the invaded range? 2/What are their geographic and host-based distributions? 3/How closely related are they, and can their mode of origin be inferred? 4/Are there candidate ‘superclones’ and newly-evolved variants that would be valuable to monitor in time and space?

**Methods**

**Field collection**

In southeastern Australia, collections occurred in December 2007 and January 2008. The southern part of Western Australia was sampled in February 2010, while New Zealand was surveyed in February 2009. Individuals from South Africa were collected at one site in 2008 (n = 26). More than 1100 individuals were collected and genotyped from 21 sites in New Zealand and 69 sites in Australia (Table S1). All sites were on public land and sampling did not require permits. No endangered or protected species were sampled as part of this study.

Sites were selected on the basis of presence of willow taxa, and distance from other sites (usually 30 to 100 km apart). At each site, a single mature tree per willow taxon was sampled. Foliation was inspected visually for sawfly presence, and any sawfly stages were collected into 96% ethanol, to a maximum of 30 individuals per tree per site. Adults were excluded because of their high mobility, except for South Africa due to small sample size. Defoliation of willow trees (i.e. when foliage on any branch had been completely consumed irrespectively of the amount of defoliation) was noted. Willow species were identified using keys for southeastern Australia [36,37,38] and New Zealand (van Kraayenoord et al. [1995]).

**DNA extraction and microsatellite analysis**

The DNA of up to 10 N. oligospilus individuals was extracted per site per willow taxon using a salting-out DNA extraction protocol [39]. The type and amount of tissue used depended on the stage of the sawfly. For adults, DNA was extracted from the abdomen, while for larvae and pupae, the head and a portion of the thorax were used. Due to their small size, whole-first instar individuals were used.

Twenty-five microsatellite loci were used to identify multilocus genotypes (MLGs) that differ by one or more allele [26]. Fourteen of the loci were polymorphic in the Australian, New Zealand and South African samples, ten of which contributed to discrimination among genotypes. The loci that did not discriminate clones were nonetheless considered worth including (particularly given the convenience of simultaneous amplification of many loci in this system) for potential detection of rare/new variants since they are polymorphic in N. oligospilus in locations with males as well as females, and in other tenthredinid taxa [26]. Use of 25 loci should yield high resolution: previous studies have attributed clonal identity in introduced populations on the basis of many fewer loci, e.g. 5 to 7 microsatellite markers [40,41]. Individuals bearing the same 25-locus genotype will be referred to as members of the same clone, while acknowledging that even mother-daughter pairs are likely to differ by at least a few point mutations somewhere in the genome. One primer of each primer pair was labelled with infra-red dyes IRD700 or IRD800 (LI-COR Biosciences). Using the Qagen PCR multiplexing kit following the manufacturer’s instructions, 12 and 13 loci were multiplexed in 10 μl reactions, with a maximum of seven loci per channel (Multiplex A: IRD700: WF2, WF11, WF15, WF21, WF26, WF27, WF40; IRD800: WF12, WF13, WF25, WF30, WF39, WF41. Multiplex B: IRD700: WF9, WF20, WF23, WF32, WF34, WF35, WF38, IRD800: WF6, WF10, WF14, WF16, WF37). PCR products were electrophoresed on 6% acrylamide gels in LI-COR Global IR2 two-dye DNA sequencers (models 4200 and 4300). Microsatellites were viewed in LI-COR software Saga Generation 2.0, and scored visually.

**Analyses**

Genetic analyses were conducted according to sampling units based on country (Australia, New Zealand and South Africa), and within countries, regions separated by major land or water barriers between populations of N. oligospilus. The rationale for the choice of these geopolitical units is that their physical organization, economic activities and biosecurity implementation are likely to be dominant factors in the likelihood of introduction. Six regions
were defined: two in New Zealand (the North and South Islands), three in Australia (southeastern Australia; southwestern Western Australia isolated from southeastern Australia by ~1100 km of the Nullarbor Plain, and Tasmania isolated by ~400 km from the nearest part of mainland Australia by the Bass Strait), and South Africa. Genotypic diversity (G/N) was calculated for each country and region, using the number of genotypes (G) divided by the number of individuals genotyped (N). Shannon-Weaver diversity index, allelic diversity, observed (Ho) and expected heterozygosity (He) were calculated in Genalex 6.4 [42]. Shannon-Weaver diversity index [43] was expressed as δ to allow comparison based on the number of genotypes present in the populations. Departures from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium and F-statistics were calculated and assessed with exact tests in Genepop 4.0 [44]. Pair-wise FST estimates between populations were calculated based on the 25 loci with combined statistical significance calculated according to Fisher’s method as implemented in Genepop. HWE analyses were performed and expected and observed heterozygosity calculated initially for the full dataset, and then for a reduced dataset containing one individual per genotype to avoid distortions caused by multiple representatives of the same clonal genotype [40,45].

Unrooted neighbour-joining trees were constructed based on Cavalli-Sforza’s chord measure of genetic distance [46] in Populations 1.2.32 (www.bioinformatics.org/~tryphon/populations/#ancre_fonctionalites). One tree was built to depict the relationships among MLGs (inferred allele changes were mapped onto branches), and one tree depicted the population network on the basis of one individual per MLG per region. To assess genetic variance within and between countries and regions, a nested Analysis of molecular variance (AMOVA) was carried out with regions nested within countries in GenAlex 6.4 [42]. P-values were assessed with 999 random permutations.

Results
Detection and distribution of clones
The combination of 25 microsatellite markers discerned 16 different MLGs in the Australasian and South African invaded range sampled (Table 1). Of the MLGs identified, ten were found in Australia, seven of which were unique to that continent. Similarly, seven MLGs were found in New Zealand, five of which were unique, while in South Africa, one of three clones was unique (Table 1, Fig. 1). The ‘Main clone’, the most widespread MLG, occurred over most sampled areas of Australia and New Zealand and was also present in South Africa (Figs. 1, 2, 3). It represented 65.7% of all individuals genotyped. It was, however, absent from Western Australia. The second most widespread MLG, the ‘North clone’ comprised 24.9% of individuals. It encompassed the northern part of southeastern Australia as well as most of Western Australia, although it was present in low proportion at several other locations (e.g. one site in Tasmania) (Fig. 3). It was also found on North and South Islands of New Zealand. It appeared to be spreading, because it was found in high proportions at some sites but smaller numbers in surrounding sites (Fig. 2). Although both clones were widespread, they rarely overlapped within a site.

Other than ‘Main’ and ‘North’ clones, most MLGs were localized and had low frequencies. Three exceptions were clone Aus1, which was found in several sites in Western Australia around the port city of Albany and was unique to this region, and Aus2 and Aus3 which were common locally (Table 1, Fig. 2 and 3). Clone Aus2 occurred at one site, along with the Main clone. Clone Aus3 occurred at two isolated sites, where it was the only clone present (Table 1, Fig. 3). Other MLGs were usually represented by one individual per site. While two MLGs were found in more than one site (Aus2, and Aus4), other MLGs were unique to a single site. Locally common MLGs were found on all willow taxa within a site. Few statements about distributions can be made for South Africa since all individuals were sampled at one site. However, one rare MLG was present in South Africa and South Australia (Aus5).

The two most widespread MLGs, ‘Main’ and ‘North’ clones were found on all willow taxa sampled showing no obvious host specialization. Similarly, locally abundant MLGs Aus1 and Aus2 were found on all the willow taxa present at the sites where they occurred. Only the ‘Main’ ‘North’ and Aus1 MLGs have been found at sites where willows suffered defoliation.

Clone relatedness and genotypic diversity
The 16 MLGs sampled were extremely similar at their 25-locus genotypes: they differed by a maximum of 3 alleles and 3 loci (Table 1). Although other interpretations are possible, the data are most parsimoniously consistent with most or all of the 15 MLGs being derived by mutation from the most common and widespread Main MLG. On an unrooted neighbour-joining tree of the MLGs, ‘Main’ is the most interior, and from it branch eight individual MLGs differing from Main each by a single allele difference (Fig. 4). Another three branches from Main each contain two or three MLGs linked by progressive single differences. One of the multi-MLG branches was limited in distribution to New Zealand (NZ1 and NZ2). The other two branches were not geographically restricted; one encompassed two localized MLGs found in Australia (Aus5 from Western Australia, Aus7 from Southeastern Australia) and also ‘North’, found in Australia and New Zealand, while the last multi-MLG branch spans Aus1 from Western Australia, and SA from South Africa. While the tree–building process did not allow reticulation, none is necessary to explain the data: MLG relationships can be depicted by simple sequential changes without homoplasy or ambiguity.

Overall, the three countries and regions had low genetic diversity based on genotypic diversity, δH and allelic diversity (Table 2). Shannon-Weaver diversity index was extremely similar for the three countries and regions, between 1.35 and 1.37. Observed heterozygosity and allelic diversity were equally low in the regions, respectively ranging from 0.42 to 0.44 and 1.48 to 1.68. There were frequent deviations from HWE in the direction of heterozygote excess, a common property of microsatellite data sets from obligate parthenogens proposed to diversify by mutation [47] (Table 2). The number of private alleles was low: New Zealand had three private alleles, while Australia had four and South Africa had none (the latter result likely contributed to by small sample size).

Low genetic differentiation among countries and regions
FST for all country or region pairs were low, ranging from less than 0.001 to 0.05. All pairwise comparisons showed that sampling locations (countries or regions) did not differ significantly in allele frequencies (p>0.05). According to the AMOVA, most of the molecular variance, 88%, was attributed to within-region effect, while only 12% was attributed to between-region effects and less than 1% to between-country effects (Table 3). The population tree placed the regions into two groups. The main grouping included southeastern Australia, Tasmania, the North and South Islands of New Zealand, with South Africa very slightly outside that group, and with Western Australia placed on a long branch slightly more allied to South Africa (Fig. 5), presumably owing to the close relationship of Western Australian-limited MLG Aus 1 and South Africa-limited SA.
| MLG   | Country          | Region (location) | Extent       | Solely present | Willow taxa                  | Marker | Frequency |
|-------|------------------|-------------------|--------------|----------------|-----------------------------|--------|-----------|
| Main  | Australia, SE, TAS | Widespread        | Yes/No       | All            | N/A                         | 0.657  |
| South Africa |               |                  |              |                |                             |        |
| New Zealand |            | NI, SI           | Widespread   | Yes/No         | All                         |        |
| North | Australia, SE, WA, TAS | Widespread       | Yes/No       | All            | WF2                         | 0.249  |
| New Zealand |            | NI, SI           |              |                |                             |        |
| Aus 1 | Australia       | WA (Albany)       | Localized    | Yes            | S. X sepulcralis             | WF2    | 0.39      |
|       |                  |                   |              |                | S. babylonica                |        |
|       |                  |                   |              |                | S. alba var. vitellina       |        |
|       |                  |                   |              |                | S. matsudana ‘tortuosa’      |        |
|       |                  |                   |              |                | S. humboldtiana              |        |
|       |                  |                   |              |                | S. X sepulcralis             |        |
|       |                  |                   |              |                | S. X chrysocoma              |        |
| Aus 2 | Australia       | SE (Cann River)   | Localized    | No             | S. fragilis/rubens          | WF2    | 0.20      |
|       |                  |                   |              |                | S. alba var. vitellina       |        |
|       |                  |                   |              |                | S. humboldtiana              |        |
| Aus 3 | Australia       | SE (Scone)        | Localized    | Yes            | S. X sepulcralis             | WF30   | 0.015     |
| Aus 4 | Australia       | SE (Junee)        | Localized    | No             | S. babylonica                | WF32   | 0.003     |
| Aus 5 | Australia       | WA (Bunbury)      | Localized    | No             | S. babylonica                | WF2    | 0.001     |
| Aus 6 | Australia       | SE (Williamstown) | Localized    | No             | S. fragilis/rubens          | WF35   | 0.003     |
| South Africa |            |                  |              |                |                             |        |
| Aus 7 | Australia       | SE (Armidale)     | Localized    | No             | S. alba var. vitellina       | WF2    | 0.001     |
| Aus 8 | Australia       | SE (Eskdale)      | Localized    | No             | S. fragilis/rubens          | WF20   | 0.001     |
| SA    | South Africa    |                   | n/a          | No             | n/a                         | WF40   | 0.003     |
| NZ 1  | New Zealand     | SI (Picton)       | Localized    | No             | S. fragilis/rubens          | WF13   | 0.008     |
| NZ 2  | New Zealand     | SI (Picton)       | Localized    | No             | S. fragilis/rubens          | WF13   | 0.001     |
| NZ 3  | New Zealand     | NI (Hamilton)     | Localized    | No             | S. fragilis/rubens          | WF40   | 0.001     |
| NZ 4  | New Zealand     | NI (Manurewa)     | Localized    | No             | S. alba var. vitellina       | WF25   | 0.001     |
| NZ 5  | New Zealand     | NI (Rotorua)      | Localized    | No             | S. fragilis/rubens          | WF21   | 0.001     |

Table 1. Nematus oligospilus multilocus genotypes (MLGs) with country, location and region (SE: Southeastern Australia, WA: Western Australia, TAS: Tasmania, SI: South Island, NI: North Island) where they occur, extent (widespread or localized), solely present (yes: single MLG present within sites, no: MLG found with other MLGs within sites), marker: the identity of the microsatellite locus showing differences when compared to Main MLG, and the frequency of each MLG.
Figure 1. Frequency of multilocus genotypes (MLGs) of *Nematus oligospilus* in South Africa, Australia and New Zealand. Each circle represents a population and relative frequency of each MLG within the population. Each color represents a different MLG. doi:10.1371/journal.pone.0097744.g001

Figure 2. Frequency of multilocus genotypes (MLGs) of *Nematus oligospilus* in New Zealand. Each circle represents a location sampled and the relative frequency of each MLG within the site. Each color represents a different MLG. doi:10.1371/journal.pone.0097744.g002
Discussion

Clone distribution and relatedness

*Nematus oligospilus* is a very successful parthenogenetic invasive organism with low genotypic variation, and two extremely successful genotypes. The ‘Main’ MLG has colonized three continents and was present in all regions studied except Western Australia, while the ‘North’ MLG was widespread in Australia, and New Zealand. They were both found on all willow taxa present, indicating little host specialization. This, coupled with their wide geographic distribution and the very high proportion of individuals sampled belonging to them, means that they fit published definitions of ‘superclones’: genotypes dominating populations over large areas; for example: [41,48] and in some invertebrates such as aphids [21,40,41,48]. Our data are unusual in that they are particularly detailed (25 loci compared to as few as five microsatellites in similar studies [41]) and samples animals from early in the invasion process. The widespread distribution of the two very common MLGs, their wide host range and the fact they were found singly at sites where willows had been defoliated may indicate that characteristics of these clones contribute to early invasion success. Our study expands the published literature to encompass highly-resolving genotypic patterns in a representative of the family Tenthredinidae, a dominant component of the Hymenopteran parthenogenetic fauna [12].

Clones were closely related, suggesting that the number of introductions into the Southern Hemisphere has been few, except perhaps if they came from a stock of unusually limited diversity.

Figure 3. Frequency of multilocus genotypes (MLGs) of *Nematus oligospilus* in Australia. Each circle represents a location sampled and the relative frequency of each MLG within the site. Each color represents a different MLG. a) southeastern Australia and Tasmania b) Western Australia. doi:10.1371/journal.pone.0097744.g003
We infer on the basis of the network of MLGs, with support from geographic and numerical distributions, that many of the clones are descended by mutation from an early invader (very likely the ‘Main’ clone). For example, some of the rarer clones seem to have arisen in situ, as they occurred in very low proportions at sites where a common MLG predominated and differed from it at only a single allele at a single locus. Following introduction in new environments, clonal lineage changes can occur through mutations as observed in other studies of invertebrates and plants [4] and [11,49,50]. The dominance of localized MLGs could be due to being favoured by environmental conditions. Conclusive determination of whether these MLGs were introduced separately or have arisen in situ would require further work, but given their geographic abundance distributions and heterozygous excess (a characteristic of mutational divergence in clones, [47]), we currently favour the latter explanation. Possibly, rarer MLGs have been or will be favoured by environmental conditions; such a process could explain the presence of some localized MLGs dominating some site. However, the present paper presents one of the few cases globally where such a process could be inferred, and so N. oligospilus and the newly-available genetic tools for it offer many possibilities to test specific hypotheses concerning the success of clonal invaders [51,52].

**Introduction and dispersal in the landscape**

The native range source of the N. oligospilus that have invaded the Southern Hemisphere has not yet been resolved, and genetic data can contribute to clarification of relationships of these insects around the world [29,35]. The modest number of extremely closely-related clones, all derivable from the ‘Main’ clone by very few mutations, indicates a small number of introductions from a single source. All Australasian and the limited number of South African individuals assayed are possibly derived from a single founding clone. However within the sampled invaded range, multiple introductions may have occurred. For example, the unique clone in Western Australia (‘Aus1’) is more closely allied to a South African MLG than it is to Australian clones (although the relationships are all extremely close). Thus Aus1 may represent an independent introduction into Australia, quite possibly from South Africa. Many unwanted introductions to Western Australia have occurred from South Africa, including many plants and invertebrates such as the African black beetle, now considered

**Host association**

Host plants can provide selection pressures that lead to specialization of herbivore populations [53,54]. While clonal specialization has been observed in some invading aphids [16,45], the widespread MLGs of N. oligospilus did not depend on association with specific willow taxa, indicating that this species may be a host generalist. However, in the field, when several willow hosts are present, population densities of N. oligospilus are constantly higher on some host taxa than others [35]. The less favoured hosts may be more challenging and exert higher selection on N. oligospilus and increase the chance for mutations to spread. In a study on Sitobion aphids, although no host specialization was found, rarer clones had enhanced performance on the most chemically defended host plants, and it was hypothesized that rarer clones were relatively favoured partly because of the presence of chemical defences [55]. Due to the low numbers of the rarer clones, this could not be assessed in this study, but warrants future investigation.

Figure 4. Unrooted neighbour-joining tree based on Cavalli-Sforza chord distance depicting relationships between different multilocus genotypes of *Nematus oligospilus*. Under the assumption that multilocus genotypes (MLGs) more interior in the network are the ancestors of more exterior ones on the same branch, inferred mutational changes are depicted on the branches, e.g. WF21 (337–333) indicates that MLG NZ5 could have arisen most parsimoniously by a 4 base pair deletion of the Wf21337 allele from an ancestor with the genotype of Main MLG. doi:10.1371/journal.pone.0097744.g004

---

**Superclones May Explain Invasion Success**

PLOS ONE | www.plosone.org 7 June 2014 | Volume 9 | Issue 6 | e97744
an important pest [56,57]. An independent introduction into Western Australia is supported by reports of sawflies first being seen there five years later than in the rest of Australia (2004) (F. Ede, unpublished).

Sawflies in general are not strong flyers [58]. Therefore, self-dispersal could occur over limited areas but is unlikely over large water- and land-barriers. For example, it is highly unlikely that *N. oligospilus* flew from South Africa to Australasia, although we cannot rule out transport by wind. Similarly, it seems improbable that ‘North’ MLG found in southeastern Australia and Western Australia dispersed unassisted across 1100 km of the Nullarbor Plain (arid habitat lacking willow hosts), or that the single individual of the ‘North’ clone sampled in Tasmania self-dispersed to the location of its capture close to Launceston airport. Most of the dispersal seen between regions is more likely due to human-mediated jump dispersal, which is also a major cause of long-dispersal in many other invasive species; for example: [59,60].

When ready to pupate, larvae move or drop to the ground, spinning cocoons on vegetation or in soil litter. In high infestation areas, cocoons can be found on any surface available including outdoor furniture and cars (Caron, pers. obs.). Cocoons could therefore easily be transported inadvertently for long distances. Furthermore, in at least a few cases people have moved sawflies deliberately in the hope of controlling willow infestations (F. Ede, pers. obs.). Human-related dispersal of invasive organisms is common and observed in other introduced sawflies and other parthenogenetic pests such as grape phylloxera (*Daktulosphaira vitifoliae* Fitch), in which dispersal among localities is due solely to human-mediated movements of infected plants [61]. However, self-dispersal plausibly explains much of the spread of the Main Table 2. Summary statistics for *Nematus oligospilus* by country (N: sample size, G: number of genotypes present, G/N: genotype diversity, $H'$: Shannon-Weaver diversity index, No. alleles: total no. of alleles over 25 loci, allelic diversity, Ho: observed heterozygosity, He: expected heterozygosity, P excess: heterozygosity excess, *n/s*: non-significant (p>0.05), *: significant (p<0.05) (He, Ho, P and $F_S$ were calculated using one individual per genotype).

|                | Australia | New Zealand | South Africa |
|----------------|-----------|-------------|--------------|
|                | WA        | SE          | Tas          | All          | North | South | All   | South |
| N              | 99        | 760         | 53           | 911          | 100   | 119   | 219   | 26    |
| No. genotypes  | 3         | 8           | 2            | 10           | 5     | 4     | 7     | 3     |
| G/N            | 0.03      | 0.01        | 0.04         | 0.01         | 0.05  | 0.03  | 0.03  | 0.11  |
| $H'$           | 1.36      | 1.36        | 1.35         | 1.36         | 1.36  | 1.37  | 1.36  | 1.37  |
| No. alleles    | 37        | 40          | 37           | 43           | 38    | 37    | 39    | 38    |
| Allelic diversity | 1.48   | 1.60        | 1.48         | 1.68         | 1.52  | 1.48  | 1.56  | 1.52  |
| Private alleles| 1         | 3           | 0            | 4            | 2     | 1     | 3     | 0     |
| Ho             | 0.440     | 0.420       | 0.420        | 0.420        | 0.440 | 0.440 | 0.440 | 0.440 |
| He             | 0.233     | 0.221       | 0.215        | 0.229        | 0.242 | 0.224 | 0.240 | 0.229 |
| P excess       | *         | *           | *            | *            | *     | *     | *     | *     |
| Multilocus $F_S$ |        | 0.879     | 0.820        | 0.909        | 0.814 | 0.886 | 0.808 | 0.805 |

Figure 5. Unrooted neighbour-joining tree based on Cavalli-Sforza chord distance depicting relationships between different regions where *Nematus oligospilus* occurs in the Southern Hemisphere (SE: Southeastern, A: Australia, NZ: New Zealand) on the basis of the multilocus genotypes (MLGs) present in each region.

Superclones May Explain Invasion Success

We thank Anne Ede for supplying *Nematus oligospilus* from various parts of the world for this study. We are grateful to thecolleagues who have allowed us to obtain material from their collections. Permission to publish this paper has been obtained from all authors.
and North MLG at smaller spatial scales within southeastern Australia and New Zealand.

Conclusions

Two dominant MLGs (or superclones) of *N. oligospilus* occur in the Southern Hemisphere invasive range. Empirically these superclones are highly successful invaders, comprising most of the spread of *N. oligospilus* through the colonized range. Whether this success persists over longer periods remains to be tested. Clones with broader preferences and tolerances may be better at colonizing new areas, but may not be as good at competing [63]. Thus we expect invasions to be dynamic in their demogenetics: for example, one or a few genetic variants can be closely associated with dispersal ability but antagonistic to reproductive success [64]. For *N. oligospilus*, new MLGs seem to have arisen *in situ* within the Southern Hemisphere. Although two main MLGs now dominate, new and rare MLGs may be favoured following changes in environmental conditions and as non-equilibria such as founder effects dissipate [3]. Furthermore, additional introductions of distinctive MLGs to the introduced population could provide new impetus to invasion, as seen with the multiple introductions of specialised clones of the pea aphid [16]. Few introductions seem to have occurred in the present case, the extreme success of *N. oligospilus* in the Southern Hemisphere highlights the importance of biosecurity. The introduction of a single individual capable of reproducing asexually and pre-adapted to the conditions of the new environment could lead to a successful invasion.

We propose that *Nematus oligospilus* is a valuable model in invasion genetics. Data and tools have been established early during invasion of significant sections of the globe. We encourage repeated and broad sampling of the invaded range and potential native range to help infer the mode and location of generation of MLGs. Coupled with detailed studies of the attributes of tractable individual clones, this can greatly add to the understanding of invasion genetics of clonal pests.

Supporting Information

Table S1  Sites and willow taxa surveyed. N: number of *Nematus oligospilus* genotyped.

(DOCX)

Acknowledgments

We would like to thank C. Eardley for samples, G. Perdomo, T. Hunt, D. Clements and T. Bramwell for technical assistance, T. Draper, M. Norgate, A. Pavlova, K. Robertson and C. Schmuki for useful discussions and G. Stone, P.W. Price and two anonymous reviewers for comments on an earlier version of the manuscript.

Author Contributions

Conceived and designed the experiments: VC FJE PS. Performed the experiments: VC. Analyzed the data: VC. Contributed reagents/materials/analysis tools: VC FJE PS. Wrote the paper: VC FJE PS.

References

1. Kolbe JJ, Glor RE, Schettino JR, Lara AC, Larson A, et al. (2007) Multiple sources, admixture, and genetic variation in introduced Anolis lizard populations. Conservation Biology 21: 1612–1625.
2. Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends in Ecology and Evolution 22: 454–464.
3. Dlugosh KM, Parker DM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17: 431–449.
4. Perez JE, Nacchio M, Alloni C, Munoz C (2006) The biology of invasions: The genetic adaptation paradox. Biological Invasions 8: 1115–1121.
5. Puillandre N, Dupas S, Dangles O, Zeddam JL, Capdevielle-Dulac C, et al. (2007) Genetic bottlenecks in invasive species: the potato tuber moth adds to the list. Biological Invasions 10: 319–333.
6. Colautti RI, Manca M, Viljanen M, Ketelaars HAM, Burgi H, et al. (2005) Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsatellites. Molecular ecology 14: 1689–1697.
7. Schmid-Hempel P, Schmid-Hempel R, Brunner PC, Seeman OD, Allen GR (2007) Invasion success of the bumblebee, Bombus terrestris, despite a drastic genetic bottleneck. Heredity 99: 414–422.
8. Pichancourt J-B, van Klinken RD (2012) Phenotypic plasticity influences the size, shape and dynamics of the geographic distribution of an invasive plant. PLoS ONE 7: 9.
9. Durka W, Bossdorf O, Prati D, Auge H (2005) Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata, Brassicaceae*) to North America. Molecular ecology 14: 1697–1706.
10. Marrs RA, Sforza R, Hulbauer RA (2008) When invasion increases population genetic structure: a study with *Centarea diffusa*. Biological Invasions 10: 561–572.
11. Wilson ACC, Sumnucks P, Hales DF (1999) Microevolution, low clonal diversity and genetic affinities of parthenogenetic Sitobion aphids in New Zealand. Molecular Ecology 8: 1653–1666.
12. Hoffmann AA, Reynolds KT, Nash MA, Weeks AR (2008) A high incidence of parthenogenesis in agricultural pests. Proceedings of the Royal Society B: Biological Sciences 275: 2473–2481.
13. Sumnucks P, Chisholm D, Turak E, Hales DF (1998) Evolution of an ecological trait in parthenogenetic Sitobion aphids. Heredity 81: 638–647.
14. Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2006) Adaptive evolution in invasive species. Trends in Plant Science 11: 298–294.
15. Dybdahl MF, Kane SL (2005) Adaptation vs. phenotypic plasticity in the success of a clonal invader Ecology 86: 1592–1601.
16. Peccei J, Figueroa CC, Silva AX, Ramirez CC, Mieuzet L, et al. (2008) Host range expansion of an introduced insect pest through multiple colonizations of specialized clones. Molecular Ecology 17: 4606–4618.
17. Sumnucks P, England PR, Taylor AC, Hales DF (1996) Microsatellite and chromosome evolution of parthenogenetic Sitobion aphids in Australia. Genetics 144: 747–756.
18. Moran NA (1992) The evolution of aphid life cycles. Annual Review of Entomology 37: 321–348.
19. Wilson ACC, Sumnucks P (2006) The genetic outcomes of sex and recombination in long-term functionally parthenogenetic lineages of Australian Sitobion aphids. Genetical Research 87: 175–183.
20. Weeks AR, Hoffmann AA (1998) Intense selection of mite clones in a heterogeneous environment. Evolution 52: 1295–1333
21. Figueres CC, Simon JC, Le Gallic JF, Prunier-Leterme N, Briones LM, et al. (2003) Genetic structure and clonal diversity of an introduced pest in Chile, the cereal aphid Sitobion avenae. Heredity 95: 24–33.
22. Zhang YY, Zhang DY, Barrett SC (2010) Genetic uniformity characterizes the invasive spread of water hyacinth (Eichhornia crassipes), a clonal aquatic plant. Molecular ecology 19: 1774–1786.
23. Ruddle KG, Gallo L, Marchelli P, Mosner E, Lirpetl S, et al. (2011) Wide spread invasion without sexual reproduction? A case study on European willows in Patagonia, Argentina. Biological Invasions 13: 45–54.
24. Wilson ACC, Surnucks P, Hales DF (2003) Heritable genetic variation and potential for adaptive evolution in assexual aphids (Aphididae). Biological Journal of the Linnean Society 79: 115–133.
25. Simon JC, Rüpe C, Surnucks P (2002) Ecology and evolution of sex in aphids. Trends in Ecology and Evolution 17: 34–39.
26. Cavanagh AD, Eardley CD (1995) A recently introduced sawfly, Nematus oligospilus, Bulletin of Entomological Research 103: 74–80.
27. Liston AD (1995) Compendium of European sawflies. Göttingen: Chalastos Entomologist 20: 51–54.
28. Smith DR (1979) Suborder Symphyta. In: Krombein KV, Hurd PDJ, Smith DR, Burke BD, editors. Catalog of Hymenoptera in America North of Mexico. Washington: Smithsonian Institution Press. pp. 3–137.
29. Naumann ID, Williams MA, Schmidt S (2002) Synopsis of the Tenthredinidae (Hymenoptera) in Australia, including two newly recorded, introduced sawfly species associated with willows (Salix spp.). Australian Journal of Entomology 41: 1–6.
30. Dapoto G, Giganti H (1994) Biogeografia de Nematus deaustai Smith (Hymenoptera: Tenthredinidae: Nematae) in las provincias de Río Negro y Neuquen (Argentina). Boletín 15: 27–32.
31. Koch F, Smith DR (2000) Nematus oligospilus Förster (Hymenoptera : Tenthredinidae), an introduced willow sawfly in the Southern Hemisphere. Proceedings of the Entomological Society of Washington 102: 292–300.
32. Urban A, Earlley CD (1995) A recently introduced sawfly, Nematus oligospilus Förster (Hymenoptera: Tenthredinidae), that defoliates willows in southern Africa. African Entomology 3: 23–27.
33. Berry JA (1997) Nematus oligospilus (Hymenoptera:Tenthredinidae), a recently introduced sawfly defoliating willows in New Zealand. New Zealand Entomologist 20: 51–54.
34. Bruzese E, McFadyen R (2006) Arrival of the leaf-feeding willow sawfly Nematus oligospilus Förster in Australia - pest or beneficial. Plant Protection Quarterly 21: 43–44.
35. Caron V, Ede FJ, Surnucks P, O’Dowd DJ (2014) Distribution and rapid range expansion of the introduced willow sawfly Nematus oligospilus Förster (Hymenoptera: Tenthredinidae) in Australasia. Austral Entomology 53: 173–182.
36. Spencer R (1997) Horticultural flora of south-eastern Australia, Flowering plants dicotyledons-part 1. Sydney: UNSW Press. 606 p.
37. Carr GW (2003) Provisional key to naturalised Salix (Salicaceae) taxa in Australia. Ecology Australia Pty Ltd.
38. Carr GW, Walsh NG (1996) Salicaceae. In: Walsh NG, Entwisle TJ, editors. Flora of Victoria. Melbourne: Inkata Press. pp. 385–398.
39. Surnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytchrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution 13: 510–524.
40. Zepeda-Paulo FA, Simon JC, Ramirez CC, Fuentes-Contreras E, Margarito-Poulson JT, et al. (2010) The invasion route for an insect pest species: the tobacco aphid in the New World. Molecular Ecology 19: 4730–4732.
41. Harrison JS, Mondor EB (2011) Evidence for an Invasive Aphid ‘‘Superclone’’: Extremely Low Genetic Diversity in Oleander Aphid (Aphis nerii) Populations in the Southern United States. Plos one 6.
42. Beakall R, Simms PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288–295.
43. Shannon CE, Weaver W (1949) The mathematical theory of communication. Champaign: University of Illinois Press.
44. Rouset F (2008) Genepop’007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8: 103–106.
45. Surnucks P, Debarro PJ, Lushai G, Maclean N, Hales D (1997) Genetic structure of an aphid studied using microsatellites: Cycle panmorphism, flow restrictions and host specialization. Molecular Ecology 6: 1039–1073.
46. Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analytic models and estimation procedures. American Journal of Humen Genetics 19: 233–257.
47. Simon JC, Baumann S, Surnucks P, Hebert PDN, Pierre JS, et al. (1999) Reproductive mode and population genetic structure of the cereal aphid Sitobion avenae studied using phenotypic and microsatellite markers. Molecular Ecology 8: 531–545.
48. Vorburger C, Lancaster M, Surnucks P (2003) Environmentally related patterns of reproductive modes in the aphid Myzus persicae and the predominance of two ‘‘superclones’’ in Victoria, Australia. Molecular Ecology 12: 3493–3504.
49. Ahmad R, Low PS, Spencer DF, Jasieniuk M (2008) Molecular evidence for a single genetic clone of invasive Arundo donax in the United States. Aquatic Invasions 3: 113–120.
50. Weetman D, Hauser L, Carvalho GR (2002) Reconstruction of microsatellite mutation history reveals a strong and consistent deletion bias in invasive clonal snails, Patinapolis antipodana. Genetics 162: 813–822.
51. Canasande LE, Figuezaga CC, Fuentes-Contreras E, Niemeyer HM, Nespolo RF (2011) Physiological approach to explain the ecological success of ‘‘superclones’’ in aphids: interplay between detoxification enzymes, metabolism and fitness. Journal of Insect Physiology 57: 1038–1046.
52. Vorburger C, Surnucks P, Ward SA (2003) Explaining the coexistence of asceuls with their sexual progenitors: no evidence for general-purpose genotypes in obligate parthenogens of the peach-potato aphid, Myzus persicae. Ecology Letters 6: 1091–1098.
53. Via S (1999) Reproductive isolation between sympatric races of pea aphid. I. Gene flow restriction and habitat choice. Evolution 53: 1446–1457.
54. Frantz A, Plantgenet M, Mieuzet L, Simon JC (2006) Ecological specialization correlates with genotypic differentiation in sympatric host-populations of the pea aphid, Journal of Evolutionary Biology 19: 392–401.
55. Figueroa CC, Simon JC, Le Gallic JF, Prunier-Leterme N, Briones LM, et al. (2004) Effect of host defense chemicals on clonal distribution and performance of different genotypes of the cereal pest Sitobion avenae. Journal of Chemical Ecology 30: 2515–2525.
56. Fisher D, Learmouth S (2001) African black beetle in vineyards. Perh.: Department of Agriculture and Food. pp. Bulletin No. 4500 Agdex 424/1/4622.
57. Scott JK, Panetta FD (1993) Predicting the Australian weed status of southern African plants. Journal of Biogeography 20: 87–93.
58. Benson RB (1950) An introduction to the natural history of British sawflies (Hymenoptera: Symphyta). Transactions of the society for British Entomology 10: 45–134.
59. Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal. Insights from Argentine ants. Proceedings of the National Academy of Sciences of the United States of America 98: 1095–1100.
60. Muirhead JR, Leung B, van Overdijk C, Kelly DW, Nandakumar K, et al. (2006) Modelling local and long-distance dispersal of invasive emerald ash borer, Agrilus planipennis (Coleoptera) in North America. Diversity and Distributions 12: 71–79.
61. Digweed SC, MacQuarrie CJK, Langor DW, Williams DJM, Spence JR, et al. (2009) Current status of invasive alien birch-leafmining sawflies (Hymenoptera: Tenthredinidae) in Canada, with keys to species. The Canadian Entomologist 141: 201–235.
62. Vorwerk S, Forneck A (2006) Reproductive mode of grape phylloxera (Daktulosphaira vitifoliae, Homoptera : Phylloxeridae) in Europe: molecular evidence for predominantly asceual populations and a lack of gene flow between them. Genome 49: 678–687.
63. Facon B, Genton BJ, Shiyouf J, Jarne P, Estoup A, et al. (2006) A general eco-evolutionary framework for understanding bioinvasions. Trends in ecology & evolution 21: 130–135.
64. Haag CR, Saastamoinen M, Marden JH, Hanski I (2003) A candidate locus for adaptation to variation in dispersal rate in a butterfly metapopulation. Proceedings of the Royal Society B-Biological Sciences 272: 2449–2456.
