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FAST TRACK

Single-queen introductions characterize regional and local invasions by the facultatively clonal little fire ant *Wasmannia auropunctata*

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Abstract

Clonal reproduction may facilitate the spread of invasive species by reducing the minimum population size necessary for successful establishment. We used microsatellite markers to reconstruct the composition of founding populations in two regional (Central Africa and Hawaii) and 23 local (near a Gabonese oilfield) invasions of the facultatively parthenogenetic little fire ant. Central Africa had a single dominant queen clone, which appears to have initiated the regional infestation, and then produced numerous other clones by rare sexual reproduction. This interpretation of the data was also supported by the genotype of a worker from the first collection in Africa (Gabon 1913). We found only a single queen clone in Hawaii, likewise indicating a single-clone introduction, most likely from an earlier infestation in Florida. Single-clone introductions also gave rise to the vast majority (92%) of local infestations at our oilfield study site. These results suggest the unusual, largely clonal, reproductive strategy of the little fire ant may enhance its success as an invasive species. However, the occasional sexual production of novel genotypes after the initial introduction may provide genetic flexibility that overcomes shortcomings of pure clonality.

Keywords: adaptation, asexuality, jump dispersal, propagule pressure, Red Queen

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Biological invasions are a major aspect of anthropogenic global change, posing challenges for conservation, agriculture, industry, and human health (Pimentel *et al*. 2000; Gurevitch & Padilla 2004; Schlaepfer *et al*. 2005; Gilchrist & Lee 2007). Since most of the species brought to new habitats fail to successfully colonize, initial establishment is a key part of the biological invasion process, which remains poorly understood (Williamson 1996). Understanding the sources of invaders and the sizes of arriving propagules has practical relevance for their management, such as the choice of biocontrol agents (e.g. Goolsby *et al*. 2006; Morin & Edwards 2006). Generally, the size of the founding population will determine its response to changing selection pressures, evolution of novel traits and dynamic stability (Sakai *et al*. 2001; Lee 2002; Novak 2007).

For a sexually reproducing species, the establishment phase poses particular difficulties, such as finding mates and, for an outbred species, the potential for inbreeding depression. Such demographic difficulties probably doom the vast majority of potential invasions. Consequently, it is not surprising that most successful invasions occurred only after repeated introductions of 'propagules' into the novel range, and that the number or size of introductions ('propagule pressure') strongly predicts the success of invasions (Suarez *et al*. 2005; Colautti *et al*. 2006). Clonal or inbreeding species may circumvent these problems, as each propagule acts as self-contained reproductive unit (Tomlinson 1966). Thus, breeding systems may play an important role in reducing the number of propagules required for a successful introduction. Indeed, many of the most successful invaders, particularly plants, show at least some asexual reproduction in the introduced range (Baker 1965; Sakai *et al*. 2001). On the other hand, purely asexual reproduction may be a liability, since it may slow the rate

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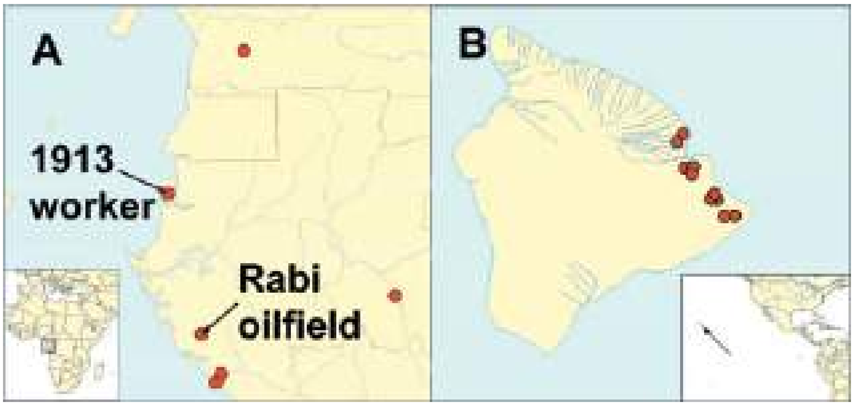

Fig. 1 Collection areas in Central Africa (A) and in Hawaii (B). In addition to isolated collections through Central Africa, we more extensively sampled 23 populations from our main field site in the Rabi oilfield (Gabon). Figure 2 shows these sampling sites in more detail. In both regions our collections circumscribe the entire invasive ranges.

of adaptation to the novel environment, and may facilitate their exploitation by predators and parasites (Burdon & Marshall 1981). In light of these considerations, empirical evidence showing the net benefit of clonal breeding systems for facilitating the introduction of successfully invading populations remains scant.

Here we use microsatellite markers to reconstruct demographics of regional and local introductions of a predominantly clonal species, the little fire ant *Wasmannia auropunctata*. In the course of the past century, this predominantly Neotropical species has colonized every continent and many Pacific archipelagos (Wetterer & Porter 2003; Jucker *et al*. 2008). Like many other invasive ants, in the introduced range *W. auropunctata* forms highly competitive super-colonies made of multiple cooperating nests (Holway *et al*. 2002; Tsutsui & Suarez 2003; Le Breton *et al*. 2004). *W. auropunctata* introductions are frequently associated with reductions of native ant and other arthropod diversity, as well as reports of attacks on vertebrates (Lubin 1984; Jourdan *et al*. 2001; Le Breton *et al*. 2003; Wetterer & Porter 2003; Walsh *et al*. 2004; Walker 2006; Mikheyev *et al*. 2008). Its dramatic effects on native species abundance and diversity have earned it a place among the World Conservation Union's top 100 most dangerous invasive species (Lowe *et al*. 2000).

Wasmannia auropunctata has a unique reproductive system, one that may facilitate its success as an invader. Workers are produced sexually, but do not themselves reproduce. Queens reproduce by parthenogenesis, which gives rise to genetically homogeneous clonal lineages. More remarkably, the haploid males also reproduce clonally (Fournier *et al*. 2005a). Although the mechanism of male clonal reproduction remains unknown, it likely involves either elimination of female genes post-fertilization, or the fertilization of anucleate ovules (Foucaud *et al*. 2007). Although this reproductive strategy provides some independence between male and queen gene pools, new clonal lineages are sometimes formed by occasional hybridization (Foucaud *et al*. 2006).

Since a single inseminated queen *W. auropunctata* may successfully initiate an invasion by producing male and female clones, asexuality may aid the establishment of new populations. This appears to have happened in New Caledonia, where a single queen was introduced and eventually gave rise to numerous sexually produced clonal lineages (Foucaud *et al*. 2006). Our goal was to determine whether this single-queen introduction was an exception, or whether such events typify little fire ant invasions. To that end, we used microsatellite marker analysis of present-day clonal distributions to reconstruct the structure of founding populations in Central Africa and Hawaii, as well as of 23 local introductions in the vicinity of a Gabonese oilfield.

Materials and methods

Field work

We conducted field work in two regions, in Central Africa (from 2006 to 2007) and in Hawaii (in 2008). Figures 1 and 2 show the locations of our sampling sites in each region. In addition to a survey across the range of the *Wasmannia auropunctata* infestation in Central Africa, we also focused on 23 populations introduced in the vicinity of the Rabi/Toucan oilfields operated by Shell-Gabon and lying within the logging concession of Compagnie des Bois du Gabon (CBG). This study area was located in lowland tropical rainforest in southwestern Gabon (1°55′S, 9°50′E). Annual rainfall averages about 2300 mm and is highly seasonal, with dry seasons from June to October and from mid-December to mid-January, although most trees are evergreen because the driest months are cool and cloudy (Laurance *et al*. 2006). Disturbance to the habitat is industrial in nature, limited to logging and petroleum extraction (Mikheyev *et al*. 2008). Using a series of security checkpoints Shell-Gabon restricts access to the oilfield to its own industrial activities and to logging by CBG.

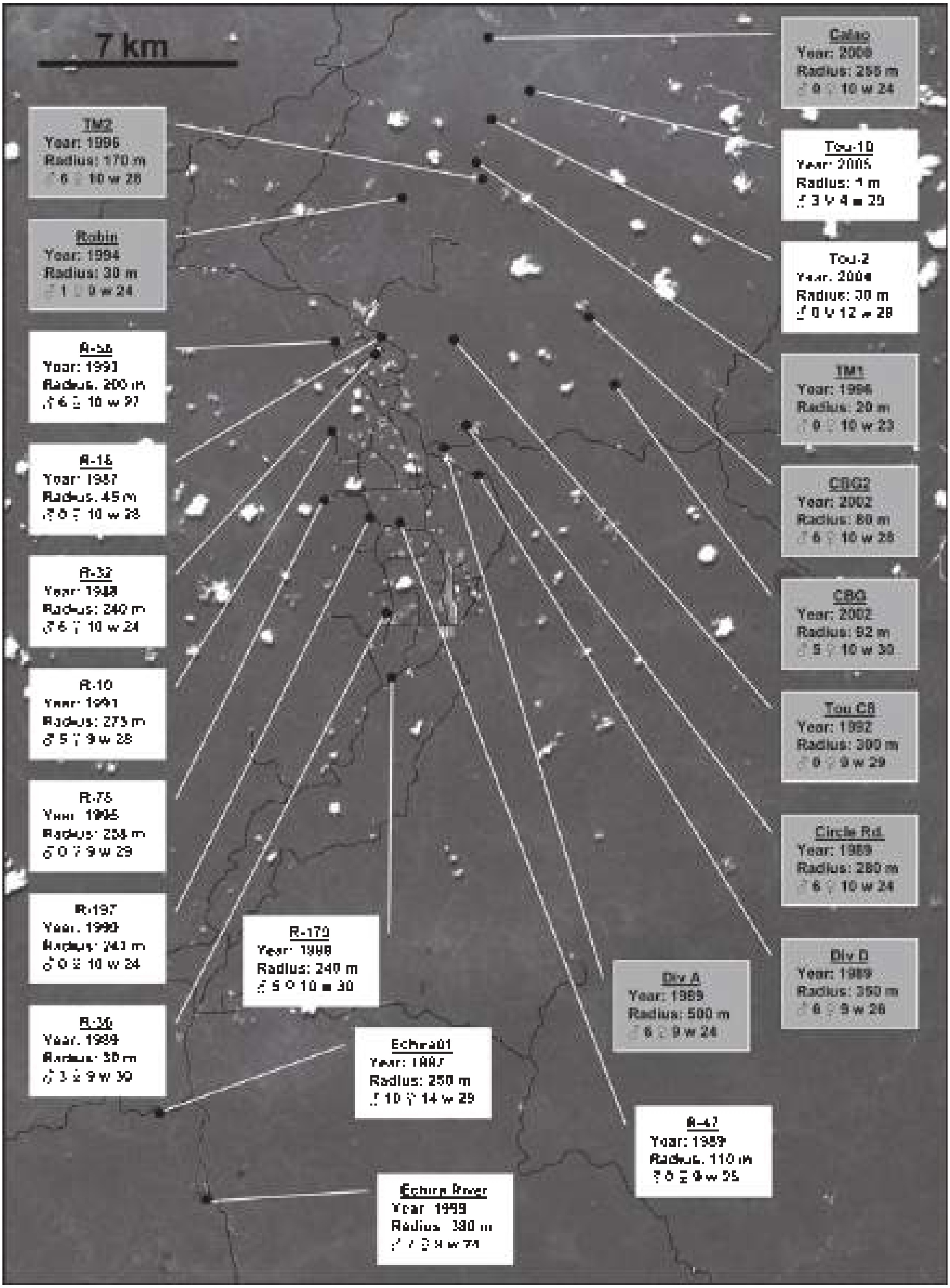

Fig. 2 Map of the Rabi oilfield with collecting sites. This satellite photograph shows the network of petroleum production and logging roads within the rainforest matrix. Populations introduced by logging (grey) and by petroleum extraction (white) were dated independently (see Materials and methods). For each site, we give the name, year of introduction, population radius, and the number of males, queens and workers genotyped.

Wasmannia auropunctata was present only in the vicinity of human-disturbed habitat. Prior work in Gabon has shown that they have a limited capacity for independent dispersal over dry land, and are primarily spread by logging and petroleum extraction (Walsh *et al*. 2004; Mikheyev *et al*. 2008). Such industrial disturbance is ideal for determining the dates of *W. auropunctata* introductions, because it occurs in a closed-off area and can be correlated with company records. Given the remote and sparsely populated location of the oilfield, industrial habitat modification is expensive, and occurs on a limited and highly controlled scale (see satellite photograph in Fig. 2). For example, after they are drilled, oil wells receive only occasional visits by small cleaning crews and no additional material is transported there. Thus, platforms are not significantly colonized by *W. auropunctata* after drilling, as shown by our recent study (Mikheyev *et al*. 2008). After a single rainy season, logging roads become impassable to vehicles and/or are immediately barricaded by CBG, severing access to remote logging sites. Thus, there is likewise little potential for further ant introductions after the initial disturbance.

We conducted extensive surveys along abandoned logging roads and in the vicinity of oil wells in order to find *W. auropunctata* populations with determinable introduction dates. As most platforms contained multiple wells, we included only those that had wells drilled within 5 years of each other. We then estimated the date of introduction as the average of the well-drilling dates, which were obtained from Shell-Gabon. The introduction of a *W. auropunctata* population around the bridge over the Echira river on the Rabi-Gamba pipeline road was dated by the bridge's construction, as the materials were brought from the highly *W. auropunctata*-infested storage yard at Rabi. The dates of *W. auropunctata* introductions on logging roads and storage yards were assumed to correspond to the period of logging activity, as all of the logging roads we surveyed were subsequently abandoned, and thereby, were rendered impassable to vehicles. Logging dates were obtained from CBG after the completion of the field surveys. CBG was not informed of the study's goals and, presumably, provided unbiased estimates. In total, the study used 12 introductions due to petroleum extraction and 11 were due to logging. They ranged in age from 6 months to 19 years (Fig. 2). The spatial extent of each population was determined using GPS, by running four orthogonal transects from the approximate centre of the infestation, usually corresponding to the probable site of introduction, such as a timber storage site or oil production platform, to well beyond the limits of the *W. auropunctata* population.

Molecular methods

For genotyping, we used the 12 microsatellite loci developed by Fournier *et al*. (2005b). We genotyped 614 workers, 256 queens and 82 males from Rabi, and 20 to 30 queens from other sites in Central Africa. In Hawaii, we genotyped 92 workers, 76 queens, 7 males and 24 sexual larvae (averaging 12 ± 8 worker and 11 ± 3 queens per site; males were found at only two sites). As it seemed likely that *W. auropunctata* may have been brought to Hawaii from the invasive population in Florida, we also analysed 4 queens, 5 males and 16 workers from an opportunistic collection made in Lake Placid, Florida. Individual workers, or the head plus thorax of an alate were ground under liquid nitrogen and then boiled for 10 min in 10% aqueous Chelex solution (300 μL for workers and 600 μL for sexuals). Two microlitres of the extract was used as template for 10 μL polymerase chain reactions (PCRs) also containing 1× reaction buffer, 1 mM dNTPs, 5 mM $MgCl_2$ and 0.1 U of ABI AmpliTaq Gold polymerase. Primer concentrations and reaction temperatures were the same as used by Fournier *et al*. (2005b). Microsatellite scoring was performed in GeneMarker (version 1.7).

Genotyping a worker from the first collection of W. auropunctata *in Africa*

We were able to obtain a single point-mounted worker from the original collection of *W. auropunctata* in Gabon in 1913 (Santschi 1914) from the Naturhistorisches Museum in Basel, Switzerland. Since no other records of *W. auropunctata* collections in Gabon exist until 1982 (Walsh *et al*. 2004, but see Bruneau de Miré 1969), we genotyped this worker to establish continuity between the present-day infestations and the first collection. In order to minimize chances of sample cross-contamination, DNA extraction and PCR preparation were carried out in a separate part of the building, using dedicated equipment and reagents. Several negative controls were set up for every reaction step and a worker from a genetically distinct population was used as a positive control. The worker was separated from the paper point and incubated at 70 °C overnight in 50 μL of extraction buffer (Goldenberger *et al*. 1995); this extraction technique leaves the specimen morphologically intact. Microsatellite DNA was amplified from 1 μL of the extract using nested PCR with two sets of primers redesigned from the loci isolated by Fournier *et al*. (2005b) (see Supporting information for their sequences). The reaction conditions were as described above, but with the addition of 0.05% Tween 20 (Goldenberger *et al*. 1995) and using a single primer pair at a time. We were able to successfully amplify loci Waur-418, Waur-1gam and Waur-1166, obtaining two alleles for each locus.

Statistical analysis

In our study of local introductions, the relationship between counts of different clones and population age was assessed

Table 1 Microsatellite genotype of dominant queen and male clones in (A) Central Africa and in (B) Hawaii. Loci specific to male and female dominant clones are indicated in bold. In Africa, frequencies of the alleles in sexually produced clones are given next to the allele length (each distinct clone counted only once). The rare alleles one stepwise mutation away from a main allele have been synonymized with that allele for ease of representation. Alleles found in the genotype of the worker from the 1913 Libreville collection are highlighted in grey

	(A) Gabon			(B) Hawaii		
Locus	Queen (♀$_0$)		Male (♂$_0$)	Queen		Male
Waur-1166	94(.36)	106(.64)	94	102	102	102
Waur-418	**99(.28)**	**113(.46)**	**97(.26)**	110	110	110
Waur-275	110	110	110	120	120	120
Waur-730	**154(.18)**	**178(.73)**	**174(.9)**	155	155	155
Waur-680	173	173	173	169	169	169
Waur-716	**183**	**197**	**null**	**183**	**197**	**183**
Waur-225	221	221	221	221	221	221
Waur-3176	226	226	226	**223**	**223**	**229**
Waur-521	**206(.35)**	**214(.63)**	**220(.01)**	229	229	229
Waur-566	**263(.94)**	**263**	**277(.06)**	**284**	**284**	**273**
Waur-1gam	**287(.38)**	**293(.08)**	**304(.55)**	288	288	290
Waur-2164	**292(.38)**	**294(.26)**	**314(.26)**	306	306	306

using Bayesian negative binomial regression with non-informative priors using WinBUGS (1.4) (Lunn *et al*. 2000). Although the number of queen samples varied slightly between sites (Fig. 2), this variability was not significantly correlated with population age ($r = 0.16$, $P = 0.46$), or the number of clones actually found ($r = 0.23$, $P = 0.29$), and was omitted from the model. The model, data and simulation results can be seen in the Supporting information. Although the same significance can be obtained using linear regression (see Supporting information), we chose the Bayesian model as it allowed a more flexible and realistic shape for the intercept parameter that describes the number of queen clones in the founding population (discrete and non-negative), making results easier to interpret. All parameter estimates are presented ± one standard deviation.

Results

Regional infestation in central Africa

Most of the queens (69%) and all of the males had the same genotype at all microsatellite loci. These dominant lineages will be referred to as ♀$_0$ and ♂$_0$. The ♀$_0$ genotype was present in all of the populations in the regional survey and 21 out of the 23 populations in the Rabi survey. A small number of clones (< 5%) had alleles 2 base pairs (bp) away from the most common allele, most likely representing stepwise mutations, or occasional scoring errors (although we rechecked the size calibration and allele calls in these rare events in order to minimize this possibility). The genotypes of all other queens had some introgression of male-specific alleles, most likely corresponding to genetic recombination between the ♂$_0$ and ♀$_0$ lineages, a phenomenon that has been previously observed in New Caledonia (Foucaud *et al*. 2006). Since most of the alleles were specific to ♀$_0$ and ♂$_0$ lineages, and ♀$_0$ was heterozygous for most alleles (Table 1), these putatively recombinant queens were missing some of the alleles found in ♂$_0$. Consequently, they could be derived from a ♀$_0$ × ♂$_0$ cross, but not vice versa, suggesting that ♀$_0$ was the initial clone introduced into Africa from the New World. The hypothesis that ♀$_0$ was the founder of the African infestation was further supported by the genotype of a worker from the first *W. auropunctata* collection in Africa, which had alleles characteristic of ♀$_0$ and ♂$_0$ (Table 1A). For the 1913 worker, in addition to the microsatellite data we were also able to sequence a 143-bp fragment of cytochrome oxidase II, using nested PCR. This nucleotide sequence is identical to that of present-day *Wasmannia auropunctata* in Gabon, which thus far has been found in only one other population in a recent worldwide survey (Mikheyev & Mueller 2007). This sequence has been submitted to GenBank under Accession no. EU642552.

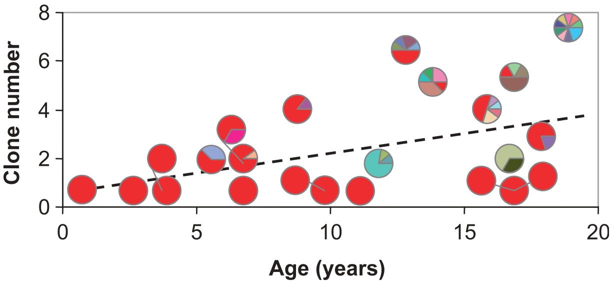

Fig. 3 Accumulation of *Wasmannia auropunctata* queen clones as a function of population age. Each clone is individually colour-coded, with the maternal clone (♀$_0$) in red. The regression of clone number on population age estimated that most infestations resulted from the introduction of one or two clone equivalents (92% and 8% posterior probability, respectively).

Local infestations in the Rabi oilfield

Paralleling the regional introduction, the populations in the Rabi oilfield were established by one (92%) or at most two (8%) queen clones (Fig. 3). The number of clones significantly increased over time, as estimated by incident rate ratio (the ratio of the number of clones at inception and after 1 year), which ranged from 1.02 to 1.10, with a mean of 1.07. This suggests that clonal richness increased over time since introduction, as new clones were sexually produced by the founding lineage. Furthermore, the recombinant queen lineages were population-specific, suggesting their origin within a population after the introduction of the maternal clone ♀$_0$, rather that a subsequent introduction from a common source.

Regional infestations in Hawaii

All queens found in Hawaii had the same genotype across all microsatellite 12 loci. Males had a clonal genotype distinct from that of the queens (Table 1B). Although relatively few males were sampled, we never saw additional alleles in the workers, indicating that these were most likely the only male genotypes found on Hawaii. One of the queens sampled from the Florida population was identical at all loci to those from Hawaii. Three were identical at every locus but one (possessing an additional 287-bp allele at locus Waur-2164). The fact that they are identical at all other loci suggests the existence of a null allele at this locus and that this null allele is shared by Hawaii queens. Although the Florida population contained at least two different male clones, neither one correspond exactly to the male clone in Hawaii, although they were genetically similar. These similarities suggest Florida as the likely source of the Hawaiian infestation.

Discussion

The finding that the genetic equivalent of a single inseminated queen gave rise to the *Wasmannia auropunctata* invasion of Hawaii parallels an earlier microsatellite study, which found that a single-queen introduction gave rise to the infestation of New Caledonia (Foucaud *et al*. 2006). This is in contrast with other well-studied ant invasions. For example, 9 to 20 fire ant (*Solenopsis invicta*) queens have established in the USA over the course of repeated introductions (Ross & Fletcher 1985; Shoemaker *et al*. 2006; Ross & Shoemaker 2008). In many haplodiploid social insects, where sex is determined by a single locus, increased homozygosity due to inbreeding causes the production of costly diploid males, which may doom a colony. For example, despite the relatively large founder population, substantial numbers of diploid males can be seen in the US fire ant population (Ross & Fletcher 1986). Although the typical number of founding queens in Argentine ant (*Linepithema humile*) introductions have not been determined, there is evidence of multiple introductions into at least some parts of the invasive range (Tsutsui *et al*. 2001). In the case of the leaf-cutting ant *Acromyrmex octospinosus*, while there is evidence that only a single female or a small group of sisters was introduced to the islands of Guadeloupe, these ants are highly polyandrous, and the introduced population has a level of genetic diversity comparable to mainland populations (Mikheyev *et al*. 2006; Mikheyev 2008). Thus, the ability of the *W. auropunctata* to overcome the short-term consequences of genetic bottlenecks, such as the production of diploid males and decreased homozygosity, likely enables it to successfully establish populations with fewer individuals than most other ant species. The widespread clonality of *W. auropunctata* explains our recent results, which showed that opportunities for colonization (oil well drilling) have played a role in the likelihood of successful of invasions in the Rabi oilfield, but had no effect on the success of subsequent expansion (Mikheyev *et al*. 2008). These finding are now explained by the genetic homogeneity of the introduced clones. Consequently, *W. auropunctata* appears more efficient than other invasive ants at spreading by anthropogenic jump dispersal, colonizing new sites at the absolute lower limit of propagule pressure.

There is considerable, if scattered, evidence that breeding systems play a role in the colonization of novel habitats. The ability to self-fertilize or reproduce clonally is well-documented in plants and is associated with many invasive plant species (Hollingsworth & Bailey 2000; Brown & Eckert 2005; Lavergne & Molofsky 2007). Similarly, the relative preponderance of inbreeding bark- and wood-boring beetle species on oceanic islands has been used to suggest that this breeding strategy may allow small founder populations to overcome potential problems associated with inbreeding depression (Jordal *et al*. 2001; Kirkendall & Jordal 2006). A facultatively parthenogenetic breeding system, such as that of *W. auropunctata*, where dominant clonality is complemented with occasional bouts of recombination may be particularly advantageous for an invasive species, since it facilitates colonization, while retaining many of the benefits of sexual reproduction, such as the generation of genetic diversity that may lead to local adaptation. This hypothesis should be tested by future research assessing the relative fitness of the founding vs. sexually produced clones. Likewise, why ♀$_0$ persists in Africa despite the ever-increasing number of daughter clones remains an intriguing mystery.

The highly invasive nature of *W. auropunctata*, which greatly expanded its range over the past century, poses a grave danger to habitat conservation in Australian, Old World and Oceanic tropical regions. Both genetic studies of unrelated *W. auropunctata* invasions to date (the present study and the earlier one in New Caledonia) have shown that a single queen introduction can initiate a full-scale invasion. This pattern is repeated after the introduction of *W. auropunctata* to a region, with local populations likewise originating from single-clone introductions. Thus, detection at the port of entry is the best way to prevent a large-scale infestation. Otherwise, an intensive eradication campaign should be undertaken as soon as an established *W. auropunctata* population is detected. Fortunately, small-scale eradication of *W. auropunctata* has been successful, but both labour-intensive and costly (Causton *et al*. 2005). However, the widespread clonality of this ant, together with limited clonal diversity in the invasive range, gives hope that biological control measures may one day be effectively employed (Burdon & Marshall 1981).

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A.S.M. is fascinated with unusual reproductive systems, and is now trying bdelloid rotifers. S.B. was an undergraduate student while he worked on this project. He will start his graduate studies in biochemistry next fall. P.C. fights on the front lines of the invasive species paradise in Hawaii.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Primers used for nested amplification of mitochondrial DNA from museum material.

Table S2 Primers used for nested amplification of microsatellite markers from museum material.