Effective population size and durability of plant resistances in the potato cyst nematode *Globodera pallida*

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Summary

- The effective size of a population is the size of an ideal population which would drift at the same rate as the real population. The balance between selection and genetic drift depends on the population size expressed as the genetically effective population size (N_e), rather than the real numbers of individuals in the population (N).
- The objectives of the present study were to estimate N_e in the potato cyst nematode *Globodera pallida* using artificial populations and to explore the link between N_e and the durability of plant resistances.
- Using a temporal method on 24 independent pairs of initial and final populations, the median N_e was 58 individuals.
- N_e is commonly lower than N but in our case the N_e/N ratio was extremely low because *G. pallida* populations deviate in structure from the assumptions of the ideal population by having unequal sex-ratios, high levels of inbreeding and a high variance in family sizes. The consequences of a low N_e could be important for the control of phytoparasitic nematodes because *G. pallida* populations will have a low capacity to adapt to changing environments unless selection intensity is very strong, which could be greatly beneficial for long-term use of plant resistances.

Keywords

Census size, Durability, Effective population size, Genetic drift, *Globodera pallida*, Nematode, Plant resistance, Selection

Introduction

Mutation, migration, selection and genetic drift determine the evolution of populations, but genetic drift has a much greater impact and selection is less effective in small than in large populations (Frankham *et al.*, 2002). When both factors are operating, selection (deterministic) predominates in large populations, while genetic drift (stochastic) predominates in small populations (Kimura *et al.*, 1963; Nei *et al.*, 1975; Gherman *et al.*, 2007). Indeed, within small populations, the random sampling of gametes due to genetic drift leads to i) random changes in allele frequencies from one generation to the next, ii) loss of genetic diversity and fixation of alleles within populations, and consequently to iii) rapid diversification among fragmented populations from the same original source. The balance between selection and genetic drift depends on the population size expressed as the genetically effective population size (N_e), rather than the real numbers of individuals in the population (N, the census size). The effective size of a population is the size of an ideal population which would drift at the same rate as the observed population (Wright, 1931). Thus, the N_e of a population is a measure of its genetic behaviour, relative to that of an ideal population, characterized by no migration, no mutation, no selection, no overlapping generations, equal sex-ratios, constant size in successive generations (i.e. on average one offspring per adult), random union of gametes, and a Poisson distribution of family sizes (Frankham *et al.*, 2002). Any characteristic of the real population that deviates from those characteristics of the ideal population will cause the effective size (N_e) to differ from the number of individuals in the population (N).

Plant pathogens impose a major constraint on food production worldwide. They are often combated with pesticides, but the need to develop more sustainable production systems fuels a trend towards a limitation of pesticide applications. Among possible alternatives to chemical control, plant resistances look promising, but can only be used in the long term and accepted in the short term if their durability is ascertained. Durability of host resistance was defined as the persistence of resistance efficiency when resistant cultivars are used over long periods, on large surfaces and in the presence of the target pathogen (Johnson, 1981, 1984): it therefore depends primarily on the rhythm of adaptive changes affecting pathogen populations in response to selection by host resistance. The speed of fixation of an advantageous allele depends on the difference in its selection coefficient from that of the other alleles (ΔS) but also on the action of genetic drift, which is influenced by the effective population size (N_e). The value of the product $N_e * \Delta S$ determines whether the population will be mainly under the

70 influence of selection or of genetic drift (Crow & Kimura, 1970; Fraser, 1972; Charlesworth,
71 2009). Consequently, the selection of virulent alleles (the virulence being defined as the ability
72 to infect a resistant host – Vanderplank, 1963; Gandon & Michalakis, 2002; Tellier & Brown,
73 2007, 2009) by resistant plants could be partly compromised by a low effective population size.

74 The effective size has been widely investigated both theoretically (Crow & Kimura,
75 1972; Nei & Tajima, 1981; Tajima & Nei, 1984; Criscione & Blouin, 2005) and experimentally
76 (Johnson *et al.*, 2004; Wang, 2005; Araki *et al.*, 2007) in a broad variety of organisms. For plant
77 pathogens, the effective size is a very important point to take into account in order to manage
78 plant resistances (e.g. Fabre *et al.*, 2012). N_e, and thus the importance of genetic drift, has been
79 explored for several plant viruses (e.g. Betancourt *et al.*, 2008; Monsion *et al.*, 2008; Zwart *et*
80 *al.*, 2011; Gutiérrez *et al.*, 2012; Fabre *et al.*, 2012, 2014) and fungi (e.g. Damgaard & Giese,
81 1996; Zhan *et al.*, 2001; Duan *et al.*, 2010; Stukenbrock *et al.*, 2011). Regarding plant parasitic
82 nematodes there is only one recent study exploring the N_e of the beet cyst nematode *Heterodera*
83 *schachtii* from wild populations, sampled on *Beta maritima* (Jan *et al.*, 2016). The objectives
84 of the present study were to estimate the N_e of the potato cyst nematode *Globodera pallida*, and
85 to explore the link between N_e and the durability of plant resistances.

86 Several methods are dedicated to estimate effective population sizes (Wang, 2005;
87 Palstra & Ruzzante, 2008; Luikart *et al.*, 2010). Methods using a single-sample estimate N_e
88 from the linkage disequilibrium and/or the heterozygote excess (Pudovkin *et al.*, 1996; Tallmon
89 *et al.*, 2008; Waples & Do, 2008), whereas temporal methods estimate N_e from the variation of
90 allelic frequencies between two temporally spaced samples. Deviations from Hardy-Weinberg
91 equilibrium due to heterozygote deficits have been recorded for three plant parasitic nematode
92 species (*Globodera pallida* - Picard *et al.*, 2004, *Heterodera schachtii* - Plantard & Porte, 2004,
93 and *Globodera tabacum* - Alenda *et al.*, 2014) and recently attributed to both consanguinity and
94 sub-structure at the plant scale (Montarry *et al.*, 2015). Those biological characteristics,
95 inbreeding and sub-structure (Wahlund effect), are known to bias single-sample estimators of
96 N_e (Zdhanova & Pudovkin, 2008; Waples & Do, 2010; Holleley *et al.*, 2014). Therefore, we
97 estimated N_e of *G. pallida* populations by using a temporal method developed by Wang (2001).

98 Rather than working with natural populations, which could sometimes harbor very low
99 genetic diversity leading to infinite estimated N_e, we decided here to work with artificial

100 populations in order to maximize the allelic diversity in the initial populations and thus to better
101 follow the variation of allelic frequencies between initial and final *G. pallida* populations.

102

103 **Materials and Methods**

104 **Biology of *Globodera pallida***

105 Nematodes are a group of worms that include free-living species such as *Caenorhabditis*
106 *elegans* as well as many parasitic species of animals and plants. Plant-parasitic nematodes are
107 major parasites that cause considerable economic losses in agriculture: worldwide crop losses
108 caused by nematodes have been estimated around $100 billion per year (Sasser & Freckman,
109 1987). *Globodera pallida* is a gonochoristic diploid organism with obligatory sexual
110 reproduction, which achieves one generation per year (Adams *et al.*, 1982). *G. pallida* is
111 probably native to the Andean Cordillera (Grenier *et al.*, 2010), the origin of its unique host
112 genus *Solanum* (Hijmans & Spooner, 2001). This obligate, sedentary endoparasite enters the
113 plant roots as second-stage juveniles (J2) and establishes a specialized feeding structure, the
114 syncytium (Jones & Northcote, 1972), which is a severe nutrient sink for the plant. Sex is
115 environmentally determined through the size of the syncytium that is induced (Sobczak &
116 Golinowski, 2011). Adult males leave the root in order to mate females. The females continue
117 to feed and when egg development is completed, they die and form a cyst, enclosing hundreds
118 of eggs, which constitute a survival stage that can stay viable for several years in soils.
119

120 **Initial nematode populations**

121 Twenty-four initial *G. pallida* populations, each composed of 100 cysts, were artificially made
122 by mixing four Peruvian populations genetically differentiated and showing high allelic
123 richness (Otuzco, Peru_252, Peru_286 and Peru_298; Picard *et al.*, 2004). Before mixing those
124 populations, the number of larvae was scored for 12 randomly chosen cysts, and a one-way
125 ANOVA showed no significant difference for the number of larvae per cyst between those four
126 populations ($F_{3,44}$ = 1.59; P = 0.21; Fig. 1). The initial census size was thus estimated by
127 multiplying the number of cysts (i.e. 100) by the mean number of larvae per cyst (i.e. 132, Fig.
128 1).

129 [Figure 1 about here.]

We mixed different numbers of cysts from the different Peruvian populations to start with allelic frequencies that differ between initial populations. Each of seven different cyst proportions was replicated three times, apart from the equal mix, which was replicated six times, for a total of 24 initial populations (Table 1). Seven initial populations (Pi_A to Pi_G), composed of 50 cysts, were also prepared in the same proportions for the estimation of initial allelic frequencies.

[Table 1 about here.]

Final nematode populations

The 24 initial *G. pallida* populations were inoculated to 24 potato plants of the susceptible potato cultivar Désirée. Because Désirée is a susceptible cultivar, there is no *a priori* reason to expect directional selection in favour of an allele. Moreover, there is also no *a priori* reason to assume that any selection affecting the allelic frequencies is acting across plants (e.g. favouring an allele in a plant and selecting against it in another plant). We have thus assumed that selection is negligible.

For each initial population, the 100 cysts were locked in a tulle bag and placed in a 13-cm pot filled with a soil mixture free of cysts (2/3 sand and 1/3 natural field soil). Tubers were then planted and covered with the same soil mixture. Plants were grown during four months in a climatic chamber regulated at 20°C with a 16h photoperiod. During that period, the monovoltine species *G. pallida* achieved only one generation. Newly formed cysts from the 24 final populations were then extracted from the soil using a Kort elutriator and stored at 4°C before genotyping. The number of newly formed cysts was counted for each final population and the number of larvae per cyst was scored for 12 randomly chosen cysts for seven final populations among the 24 (i.e. one randomly chosen population per initial proportion). The final census size was thus estimated by multiplying the mean number of cysts by the mean number of larvae per cyst.

Microsatellite genotyping

158 The 31 *G. pallida* populations (i.e. seven initial and 24 final populations) were genotyped using
159 12 neutral microsatellite markers (Gp106, Gp108, Gp109, Gp111, Gp112, Gp116, Gp117,
160 Gp118, Gp122, Gp126, Gp135 and Gp145) developed by Montarry *et al.* (2015) directly from
161 the *G. pallida* genome (Cotton *et al.*, 2014). For each population, from 26 (for Pi_E) to 40 (for
162 Pi_C) larvae, coming from distinct and randomly chosen cysts, were successfully genotyped.
163 Two multiplex panels were used to reduce the time and cost required to genotype the 1,105
164 individuals at the 12 loci.

165 DNA from single larva (i.e. one second-stage juvenile J2) was extracted following a
166 procedure using sodium hydroxide and proteinase K (Boucher *et al.*, 2013). PCR was performed
167 using a 384-well reaction module (BIO-RAD C1000) in a 5 µL volume containing 1X of Type-
168 it Microsatellite PCR kit, 0.4 µM of primer mix and 1 µL of template DNA. Cycling conditions
169 included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95
170 °C for 30 s, annealing at 57 °C for 90 s and extension at 72 °C for 30 s, followed by a final
171 extension at 60 °C for 30 min. PCR products were then diluted to 1:25 in sterile water and 3 µL
172 of this dilution were mixed with 0.05 µL of GeneScan 500 LIZ Size Standard (Applied
173 Biosystems) and 5 µL of formamide (Applied Biosystems). Analyses of PCR products were
174 conducted on ABI Prism® 3130xl sequencer (Applied Biosystems). Allele sizes were
175 determined by the automatic calling and binning module of GeneMapper v4.1 (Applied
176 Biosystems) with manual examination of irregular results. To minimize the rate of genotyping
177 errors, a second round of PCR and electrophoresis was performed for 10% of the global number
178 of individuals.

179

180 **Population genetic characteristics**

181 Genetic diversity of each nematode population was estimated through allelic richness (A_r) and
182 unbiased gene diversity (H_{nb}) (Nei, 1978). Departure from Hardy-Weinberg equilibrium was
183 tested through the F_{IS} estimation for each population. H_{nb} and F_{IS} were computed using GENETIX
184 4.05.2 (Belkhir *et al.*, 1996-2004). The statistical significance of F_{IS} values for each population
185 was tested using the allelic permutation method (1,000 permutations) implemented in GENETIX.
186 A_r was estimated on a reduced sample of 26 individuals using the rarefaction method
187 implemented in Populations 1.2.32 (Langella, 2000).

Because heterozygote deficits in cyst nematodes could be due to a Wahlund effect (i.e. sub-structure) and/or to consanguinity (Montarry *et al.*, 2015), we used the method of Overall and Nichols (2001) in order to calculate a likelihood surface for the genetic correlation due to population subdivision (θ) and the proportion of the population practicing consanguinity (C). The method, which is based on the argument that consanguinity and sub-structure generate distinctive patterns of homozygosity in multilocus data, was applied with a degree of relatedness of 1/4 (see Montarry *et al.*, 2015) to all initial and final populations showing significant heterozygote deficits. The most likely parameter combination was identified over a grid of 10,000 combinations of θ and C values, and graphs of the likelihood surface were drawn for each nematode population using the statistical software R version 3.1.1 (R Core Team, 2014).

Effective population size estimation

The temporal method developed by Wang (2001) was used to estimate N_e from the 24 independent pairs of initial and final populations of *G. pallida*. That likelihood-based method has been implemented in the MLNE 1.0 software (Wang & Whitlock, 2003).

The effect of initial populations, differing by the proportion of cysts coming from the different Peruvian populations, on N_e was tested using an ANOVA. Normality and homogeneity of variances were checked with the Shapiro-Wilk and the Levene tests, respectively, and mean values were compared with a Tukey test ($\alpha = 0.05$). The correlation between the N_e estimates and the number of newly formed cysts in each final population was tested using the Pearson's correlation test. All statistical analyses were performed using R.

Exploring the link between N_e and resistance durability

The effective population size could be important for the control of plant pathogens because the effectiveness of selection is directly linked to the effective population size: selection is less effective in small than in large populations. Indeed, the probability of fixation of an allele in a population depends on its initial frequency, its selective advantage (or disadvantage) and the effective population size. Kimura (1962) showed that the probability of fixation of allele A is given by:

$$P(A) = (1 - e^{-4.Ne.s.q}) / (1 - e^{-4.Ne.s})$$

where q is the initial frequency of allele A, s the selection coefficient and N_e the effective population size. For a neutral allele, the fixation probability equals its frequency in the population (because genetic drift favours neither allele) but for advantageous alleles, the relationship between the fixation probability and the selection coefficient depends on the effective population size.

Fixing the initial frequency of allele A at 1% (q = 0.01), the relationship between the fixation probability and the selection coefficient was explored for five N_e values, i.e. for the estimated N_e and for N_e two-, five-, 10- or 50-times higher.

Moreover, the selection coefficients exerted by four resistant potato genotypes were estimated using data from Fournet *et al.* (2013), who used these genotypes (96F.376.16, 94T.146.52, 360.96.21 and 60.96.1) to perform an experimental evolution during eight *G. pallida* generations. The selection coefficient *S* was calculated using the univariate breeder's equation (Lush, 1937): $R = h^2 * S$, where *R* is the change in mean phenotype between two generations, h^2 is the heritability, i.e. the proportion of phenotypic variance in the trait that is attributable to genetic effects. Making the assumption that the heritability of the measured trait (the number of produced females), which strongly covaries with fitness, is high, i.e. $0.8 < h^2 < 1$, resulted in a range of values for *S* for each potato genotype.

Results

Genetic characteristics of initial and final populations

As expected, genetic diversity was high for all initial populations ($0.64 < H_{nb} < 0.69$ and $6.25 < A_r < 7.34$; Table 2) and that diversity was conserved from one generation to the next, i.e. for the final populations ($0.59 < H_{nb} < 0.70$ and $5.54 < A_r < 7.14$; Table 2). All populations, except one (Pf_05), showed a significant heterozygote deficit, with F_{IS} ranging from 0.32 to 0.43 for initial populations and from 0.05 to 0.22 for final populations (Table 2). Those heterozygote deficits were due to consanguinity and substructure for the seven initial populations (Table 2 and Fig S1-A, Supporting Information) and only to consanguinity for the 23 final populations showing significant heterozygote deficits (Table 2 and Fig S1-B, Supporting Information).

[Table 2 about here.]

Estimation of effective and census population sizes

N_e ranged from 25 to 228 individuals, the mean N_e being 86 individuals and the median N_e being 58 individuals (Fig. 2).

[Figure 2 about here.]

There was a marginally significant effect of initial populations, differing by the proportion of cysts coming from the different Peruvian populations ($F_{6,16} = 2.9$; $P = 0.041$), but the comparison of means, performed with the Tukey test, was not able to identify distinct homogenous groups. Moreover, there was no significant correlation between mean N_e (calculated for each pair of initial and final populations) and the number of cysts coming from each of the four Peruvian populations (data not shown).

The number of newly formed cysts ranged from 1,060 to 3,407 with a mean (±sem) of 2,189 (±116). There was no correlation between N_e estimates and the number of newly formed cysts (Pearson's coefficient cor = -0.18; $P = 0.41$). The number of larvae per cyst, scored for seven final populations, ranged from 196 to 288 with a mean (±sem) of 235 (±12), and a one-way ANOVA showed no significant difference for the number of larvae per cyst between those seven final populations ($F_{6,77} = 0.74$; $P = 0.62$; Fig. 3). Consequently, our estimation of the final census size (N) was 514,415 larvae (2,189 newly formed cysts * 235 larvae per cyst).

[Figure 3 about here.]

Exploring the link between N_e and resistance durability

Fixing the initial frequency of an allele at 1%, results showed that for $N_e = 58$, the fixation probability was higher than 50% only for selection coefficients above 0.3, whereas for N_e two-, five-, 10- or 50-times higher, this threshold of 50% was crossed for lower selection coefficients (Fig. 4).

Using the breeder's equation, the estimation of the selection coefficients S exerted by the four resistant potato genotypes used by Fournet *et al.* (2013) showed that, for $N_e = 58$, the

fixation probability was small for the genotype 96F.376.16 ($0.08 < S < 0.10$), medium for 94T.146.52 ($0.25 < S < 0.31$) and 360.96.21 ($0.34 < S < 0.43$) and very high for 60.96.1 ($0.78 < S < 0.97$) (Fig. 4).

[Figure 4 about here.]

Discussion

This report evaluates the effective size of populations of *Globodera pallida*, the potato cyst nematode. Rather than working with natural populations, which could sometimes harbor very low genetic diversity leading to infinite estimated Ne, we decided here to work with artificial *G. pallida* populations. As expected, and because we have mixed four Peruvian populations that are genetically differentiated, heterozygote deficits observed for initial populations were very high (mean $F_{IS} = 0.39$) and due to both consanguinity and sub-structure, whereas after one generation of mating, heterozygote deficits observed for final populations were lower (mean $F_{IS} = 0.13$) and only due to consanguinity, as in natural *G. pallida* populations (Montarry *et al.*, 2015). Moreover, temporal methods assume neither migration nor selection and that the variation in allele frequencies between the samples is only due to genetic drift. Working with artificial population is thus a way to ensure the absence of migration, and allows to reduce the action of selection by using a susceptible potato cultivar. The requirement of an estimation of the generation number leads to difficulties in the evaluation of N_e for several species. For example, estimation of N_e during infection cycle of plant virus populations is quite complicated because of the lack of estimates of generation times for viruses (Fabre *et al.*, 2014). Regarding the beet cyst nematode *H. schachtii*, which is a plurivoltine species, Jan *et al.* (2016) have used two extreme estimations of the generation number. We have not that problem using the monovoltine species *G. pallida* because it performed only one generation over the experiment.

Using the likelihood-based method developed by Wang (2001), the median of the 24 N_e estimates was 58 individuals. The census size N of the initial populations was 13,200 individuals (100 cysts * 132 larvae per cyst) and our estimation of the census size of the final populations was 514,415 larvae (2,189 newly formed cysts * 235 larvae per cyst). To obtain the N_e/N ratio, we computed the harmonic mean of these two values for N as recommended by Waples (2005), yielding $\overline{N} = 25{,}740$, and thus $Ne/N \approx 2.10^{-3}$. Based on a meta-analysis, values

of N_e/N average only 10-15% (Frankham, 1995; Palstra & Ruzzante, 2008). Thus, effective population sizes are substantially lower than census sizes. For example, the threatened winter run of chinook salmon in the Sacramento River of California has about 2,000 adults, but its effective size was estimated to be only 85 (N_e/N = 0.04 - Bartley et al. 1992). N_e is thus commonly lower than N but in our case the N_e/N ratio is extremely low, close to values recorded in marine fishes (Hoarau *et al.*, 2005).

The effective size of a population is the size of an ideal population which would drift at the same rate as the observed population (Wright, 1931) and all characteristics that deviate between an ideal population and the real populations will cause the effective size (N_e) to differ from the number of individuals in the population (N). As mentioned above, some of those characteristics are similar between an ideal population and our nematode populations (i.e. no migration, no selection, no possibility for variation of N over generations and no overlapping generations) but others differ. Particularly, our real populations deviate in structure from the assumptions of the ideal population by having unequal sex-ratios and showing non random union of gametes. When larvae of different *G. pallida* populations were inoculated to susceptible potato roots in Petri dishes, the percentage of female produced was on average 60% (Fournet *et al.*, 2013). A meta-analysis showed that unequal sex-ratios have modest effects in reducing effective population sizes below actual sizes, resulting in an average reduction of 36% (Frankham, 1995). *G. pallida* populations are characterized by high levels of inbreeding, highlighted here for artificial populations (i.e. F_{IS} significantly higher than zero due to consanguinity) and previously highlighted for natural populations (Montarry *et al.*, 2015), which could also reduce effective population sizes (Charlesworth, 2009). While random mating generally sustains effective population sizes of pathogens (Barrett *et al.*, 2008), inbreeding increases the extent of genetic drift in pathogen populations, resulting in reduced N_e (Nunney & Luck, 1988). This factor on its own is however not able to explain the extremely low N_e/N ratio we observe as inbreeding can reduce effective population size by 50% at most (Charlesworth, 2009). The census size of our initial and final nematode populations has increased from 13,200 to 514,415 individuals (i.e. multiplied by 39), indicating clearly that there were several offspring produced per adult. Whether all adults of the initial populations contributed equally to the final populations is however unlikely. It has been documented in cyst nematode species of the genus *Heterodera* that both males and females mate several times, with males contributing differently to the pool of larvae (Green *et al.*, 1970; Triantaphyllou &

Esbenshade, 1990). Patterns of mitochondrial gene diversity between larvae from the same cyst in a species in which mitochondrial DNA is biparentally transmitted (Hoolahan *et al.*, 2011) support the same mating pattern for *G. pallida* (J. Ferreira de Carvalho, S. Fournet & E. J. Petit, unpublished). Using our experimental data, we estimated the variance in family sizes to range between 130 and 2,100 (see Supplementary Table S1), suggesting indeed that some individuals do not contribute at all to the next generation (Hedrick, 2005). Because we estimated N_e and N from one generation of J2 larvae to the next, these extreme figures combine both a low probability for each larvae to reach the adult stage, and a high variance in reproductive success for adults. The probability to reach adulthood can here be estimated from the ratio of twice the number of formed cysts (assuming a balanced sex-ratio) to the number of inoculated larvae, that is 2*2,189 / 13,200 = 0.33, meaning than at least 1/3 of all individuals have zero breeding success. Taking into account this proportion of non-breeders is however far from being able to explain the low N_e/N on its own (see Eq. 5c in Hedrick, 2005). Ultimately, it is the combined impact of all these factors (i.e. extreme variance in family sizes, unequal sex-ratios, and inbreeding) which could explain that the N_e/N ratio is extremely low in *Globodera pallida* populations.

The low effective population size highlighted here for the potato cyst nematode *G. pallida* is consistent with estimations performed for wild populations of the beet cyst nematode *H. schachtii*: N_e around 85 individuals with a N_e/N ratio less than 1% (Jan *et al.*, 2016). Note that because the genetic of *G. pallida* populations deviates from that of an ideal population, the effective population size well describes the genetic drift intensity but estimates poorly the number of individuals that pass on their genes through generations. That explains why we obtained more females (2,189 newly formed cysts) than the estimated N_e (58 individuals). It however appears that the effective population size of phytoparasitic cyst nematodes is lower than N_e estimates of the free living nematode *Caenorhabditis elegans* (Barrière & Felix, 2005; Sivasundar & Hey, 2005; Cutter, 2006) and of animal parasitic nematodes (e.g. for *Trichostrongylus axei* – Archie & Ezenwa, 2011).

The consequences of a low N_e could be important for the control of phytoparasitic cyst nematodes. When N_e is large, competition between individuals is fully acting, with no or little interference of random processes, and selection shapes the genetic composition of populations. Conversely, in populations with a small N_e, genetic drift, resulting in stochastic sampling of individuals that will engender the next generation, is prevalent and counters the effect of

selection. Consequently, *G. pallida* populations will have a low capacity to adapt to changing environments unless selection intensity is very strong and that could be greatly beneficial for long-term use of plant resistances (McDonald & Linde, 2002). In this paper, for the first time, we were able to determine a selection pressure threshold that allows a better risk assessment and to demonstrate that durable resistance to cyst nematodes is a truly achievable goal. Indeed, for $N_e = 58$, our results showed that the fixation probability was small for the resistant potato genotype 96F.376.16, medium for 94T.146.52 and 360.96.21 and very high for 60.96.1, showing that resistance durability could be anticipated, and that the resistance of cultivars exerting a low selection pressure on pathogen population would be durable only for pathogens showing a low N_e. It is however important to consider that in natural field populations of *G. pallida*, genotype flow have been reported (Picard *et al.*, 2004), and those genotype flow could partly compensate the impact of genetic drift (Palstra & Ruzzante, 2008). In cyst nematode, genotype flow has mainly been attributed to the passive transport of cyst through agricultural practices (Alenda *et al.*, 2014). Therefore, all agricultural management strategies that reduce genotype flow and thus promote small effective population sizes would be beneficial for the durability of plant resistances.

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Author contributions

SBV, RM, and JM performed the experiments according to a protocol elaborated jointly by SF, EG and JM. PLJ, EJP and JM analyzed the data. SF, EJP, EG and JM wrote the text and prepared the figures. All authors edited the paper and have approved the current version.

Data archival location

All data used in this article are available at datadryad.org (doi: to be completed).

Figure Legends

Fig. 1 Number of larvae per cyst for the four *G. pallida* Peruvian populations (Otuzco, Peru_252, Peru_286 and Peru_298). No significant difference was observed between those populations ($F_{3,44}$ = 1.59; P = 0.21).

Fig. 2 Histogram showing the distribution of the independent effective population sizes estimated using the Wang's method. The median N_e is indicated directly onto the box plot below the histogram.

Fig. 3 Number of larvae per cyst for the seven final *G. pallida* populations. No significant difference was observed between those populations ($F_{6,77}$ = 0.74; P = 0.62).

Fig. 4 Probability of fixation of an allele (P(A)), with an initial frequency of 1%, according to its selection coefficient (s) for a diploid population showing an effective population size (N_e) of 58 individuals and for populations showing $2N_e$ (116 individuals), $5N_e$ (290 individuals), $10N_e$ (580 individuals) and $50N_e$ (1,160 individuals). The dotted line indicates the probability of fixation of 50%. Credible intervals for selection coefficients are given as grey backgrounds for four different potato cultivars studied by Fournet et al. (2013). See text for more details.

Supporting Information

Additional supporting information may be found on the online version of this article.

Fig. S1 Likelihood surfaces showing estimated θ and C values with 95 and 99% confidence envelopes (internal and external envelopes of the highest likelihoods, visualized as grey shades, respectively) for (**A**) the seven initial *G. pallida* populations, (**B**) the 23 final *G. pallida* populations showing significant heterozygote deficits. θ and C are represented on the x-axis and y-axis, respectively.

Table S1 Estimation of the variance in family size (S^2_k) from the effective population sizes (N_e) and proportions of inbred matings (C) estimated for each final population. The computation was not possible for Pf_15 (no N_e estimate). The estimation was based on Caballero and Hill (1992, Eq. 10), with α computed from C after Ghai (1969, Eq. 17), and N = 13,200, the census size of initial populations (see Text).

627 **Table 1** Number of cysts from each of the four Peruvian *G. pallida* populations (Otuzco,
628 Peru_252, Peru_286 and Peru_298) used to construct the 24 initial populations (Pi_01 to
629 Pi_24). The estimation of the initial allelic frequencies was performed through the genotyping
630 of the seven initial populations (Pi_A to Pi_G) composed of 50 cysts mixed using the same
631 proportions.

Genotyped population	Initial population	Otuzco	Peru_252	Peru_286	Peru_298
Pi_A	Pi_01	20	30	20	30
	Pi_02	20	30	20	30
	Pi_03	20	30	20	30
Pi_B	Pi_04	30	30	20	20
	Pi_05	30	30	20	20
	Pi_06	30	30	20	20
Pi_C	Pi_07	20	30	30	20
	Pi_08	20	30	30	20
	Pi_09	20	30	30	20
Pi_D	Pi_10	30	20	20	30
	Pi_11	30	20	20	30
	Pi_12	30	20	20	30
Pi_E	Pi_13	20	20	30	30
	Pi_14	20	20	30	30
	Pi_15	20	20	30	30
Pi_F	Pi_16	30	20	30	20
	Pi_17	30	20	30	20
	Pi_18	30	20	30	20
Pi_G	Pi_19	25	25	25	25
	Pi_20	25	25	25	25
	Pi_21	25	25	25	25
	Pi_22	25	25	25	25
	Pi_23	25	25	25	25
	Pi_24	25	25	25	25

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Table 2 Number of cysts (cysts), number of genotyped individuals (n), genetic diversity (H_{nb} and A_r) and departure from Hardy-Weinberg equilibrium (F_{IS}) for each *G. pallida* population (i.e. seven artificial initial populations and 24 final populations). F_{IS} values significantly different to zero are indicated in bold. For each population showing a significant heterozygote deficit, θ and C values corresponding to the maximum-likelihood were indicated.

Population	**cysts**	**n**	**H_{nb}**	**A_r**	***F_{IS}***	***θ***	**C**
Initial populations							
Pi_A	100	39	0.68	7.23	**0.37**	0.31	0.13
Pi_B	100	38	0.68	7.23	**0.41**	0.35	0.23
Pi_C	100	40	0.65	7.34	**0.32**	0.25	0.24
Pi_D	100	39	0.64	6.25	**0.43**	0.38	0.28
Pi_E	100	26	0.65	6.57	**0.37**	0.23	0.60
Pi_F	100	38	0.69	7.30	**0.39**	0.25	0.60
Pi_G	100	36	0.68	6.90	**0.43**	0.26	0.85
Final populations							
Pf_01	2704	28	0.65	7.00	**0.14**	0.00	0.52
Pf_02	3407	33	0.66	6.51	**0.14**	0.00	0.45
Pf_03	3136	38	0.68	6.31	**0.16**	0.00	0.54
Pf_04	2227	36	0.59	6.35	**0.11**	0.03	0.35
Pf_05	2541	34	0.60	5.54	-0.01	.	.
Pf_06	2082	36	0.62	6.24	**0.13**	0.00	0.48
Pf_07	2053	37	0.67	7.14	**0.10**	0.02	0.38
Pf_08	2511	38	0.70	6.87	**0.17**	0.00	0.56
Pf_09	1459	32	0.65	6.59	**0.12**	0.00	0.46
Pf_10	2193	34	0.66	6.78	**0.18**	0.02	0.59
Pf_11	2425	37	0.63	6.27	**0.05**	0.00	0.25
Pf_12	1749	39	0.64	6.40	**0.22**	0.05	0.57
Pf_13	2893	33	0.63	6.29	**0.05**	0.00	0.18
Pf_14	1391	36	0.61	6.45	**0.13**	0.00	0.49
Pf_15	1060	35	0.68	6.77	**0.07**	0.00	0.26
Pf_16	2613	36	0.60	6.00	**0.18**	0.00	0.56

Pf_17	2161	36	0.60	5.93	**0.16**	0.00	0.50
Pf_18	1641	39	0.63	6.82	**0.22**	0.07	0.63
Pf_19	2776	34	0.60	6.37	**0.17**	0.06	0.46
Pf_20	2117	37	0.65	6.73	**0.13**	0.00	0.46
Pf_21	1753	35	0.61	6.19	**0.08**	0.00	0.31
Pf_22	1815	34	0.62	6.53	**0.21**	0.02	0.75
Pf_23	2056	36	0.61	6.69	**0.18**	0.01	0.61
Pf_24	1779	36	0.64	6.81	**0.11**	0.03	0.33

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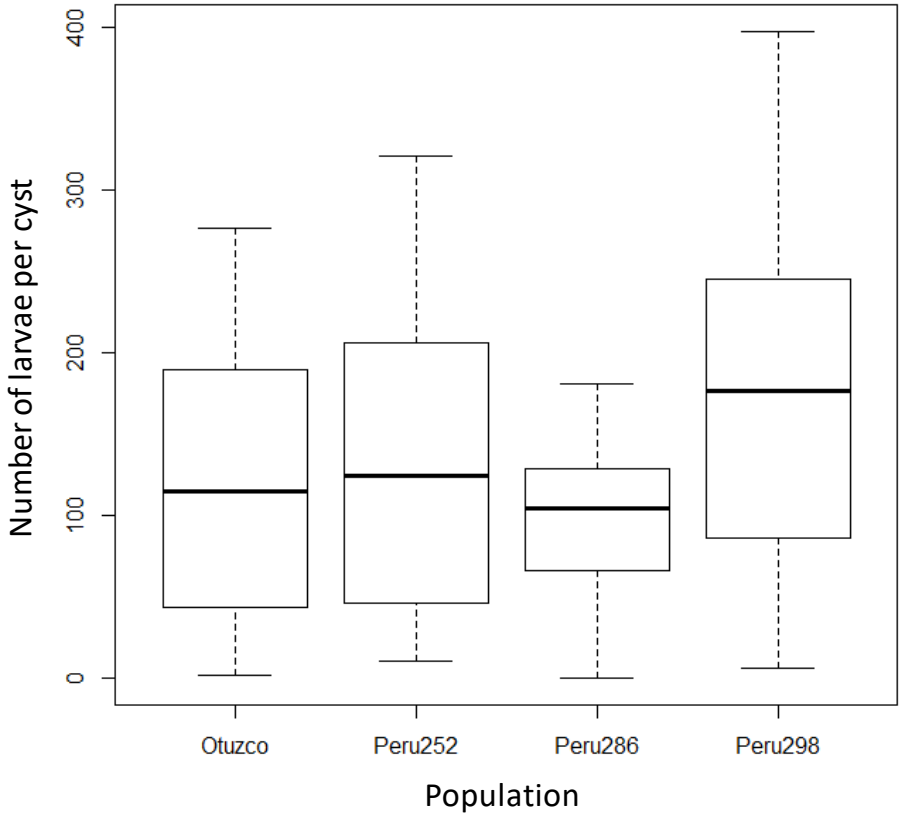
641 **Figure 1 – Montarry et al.**



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644 **Figure 2 – Montarry et al.**

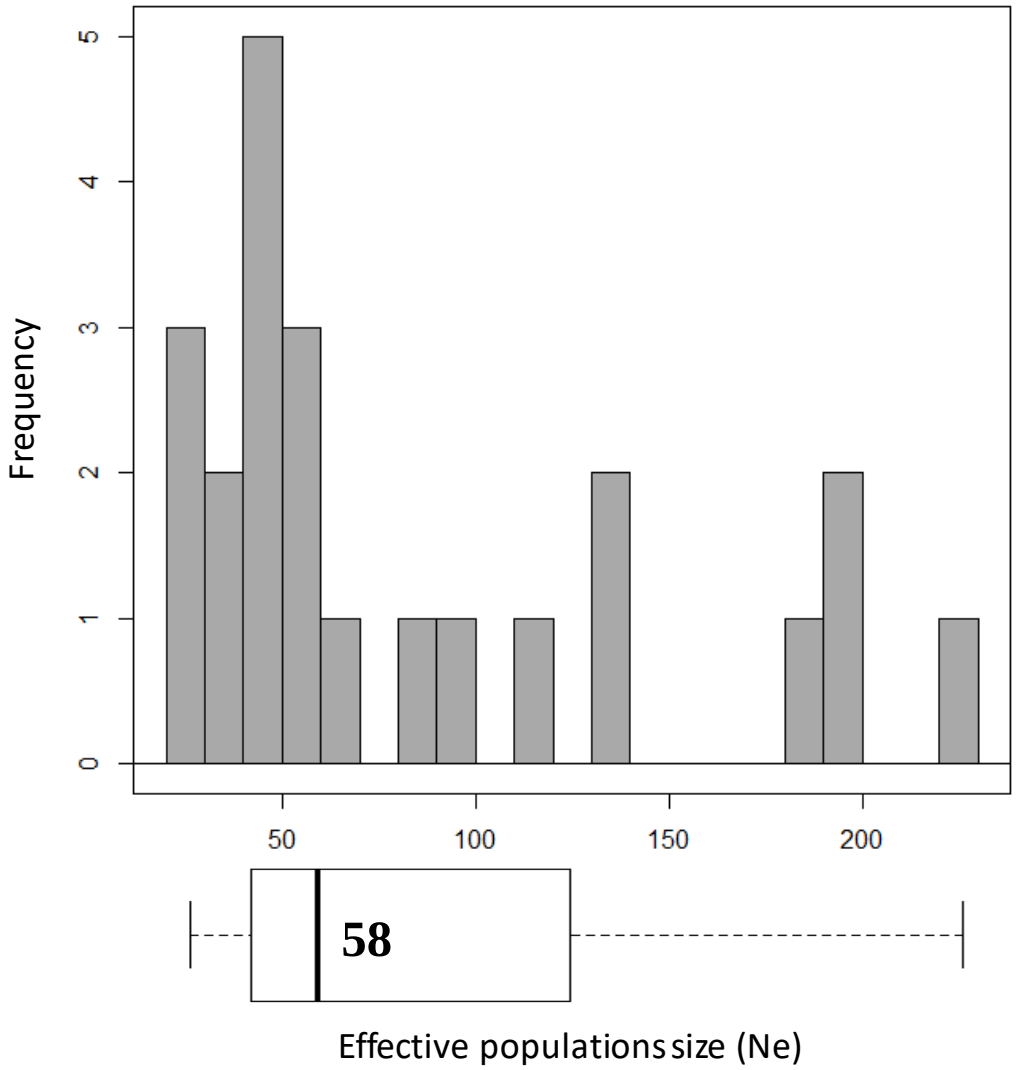

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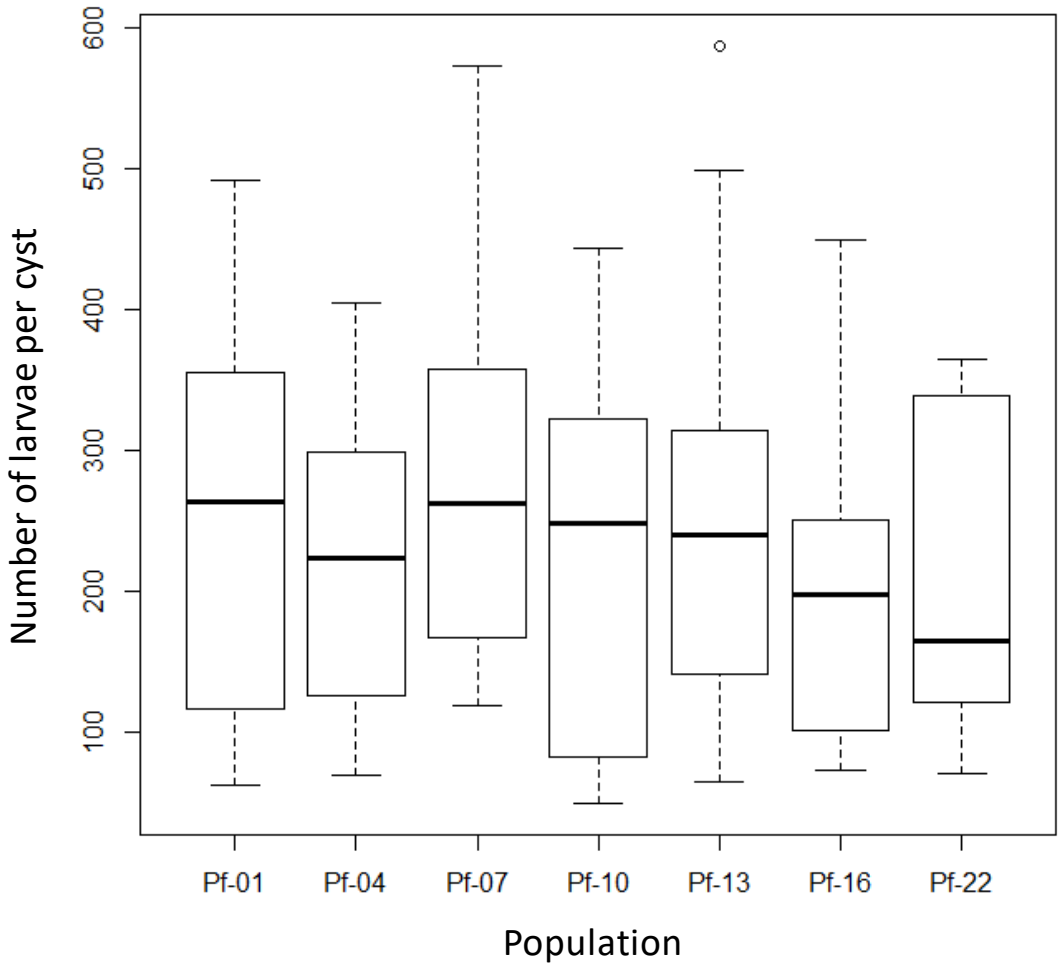
Figure 3 – Montarry et al.



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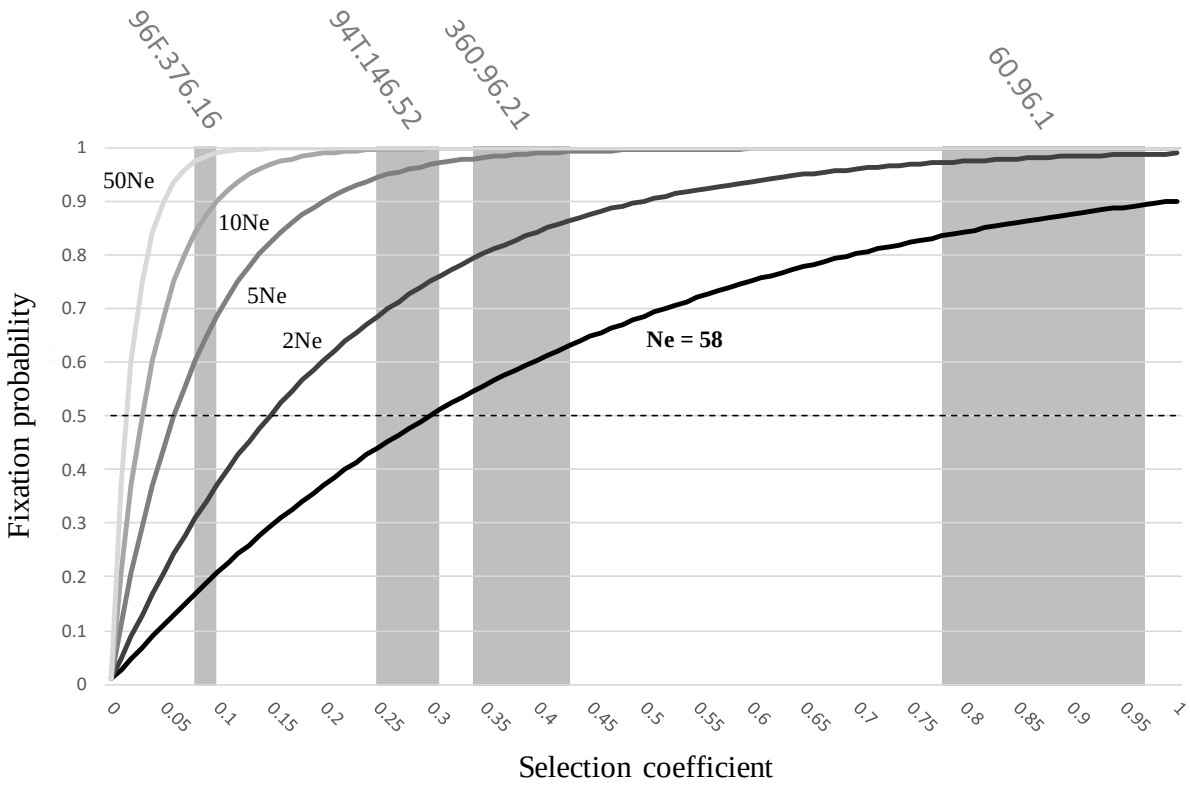

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Supporting information _ Montarry et al.

Fig. S1: Likelihood surfaces showing estimated ϑ and C values with 95 and 99% confidence envelopes (internal and external envelopes of the highest likelihoods, visualized as grey shades, respectively) for (**A**) the seven initial *G. pallida* populations, (**B**) the 23 final *G. pallida* populations showing significant heterozygote deficits. ϑ and C are represented on the x-axis and y-axis, respectively.

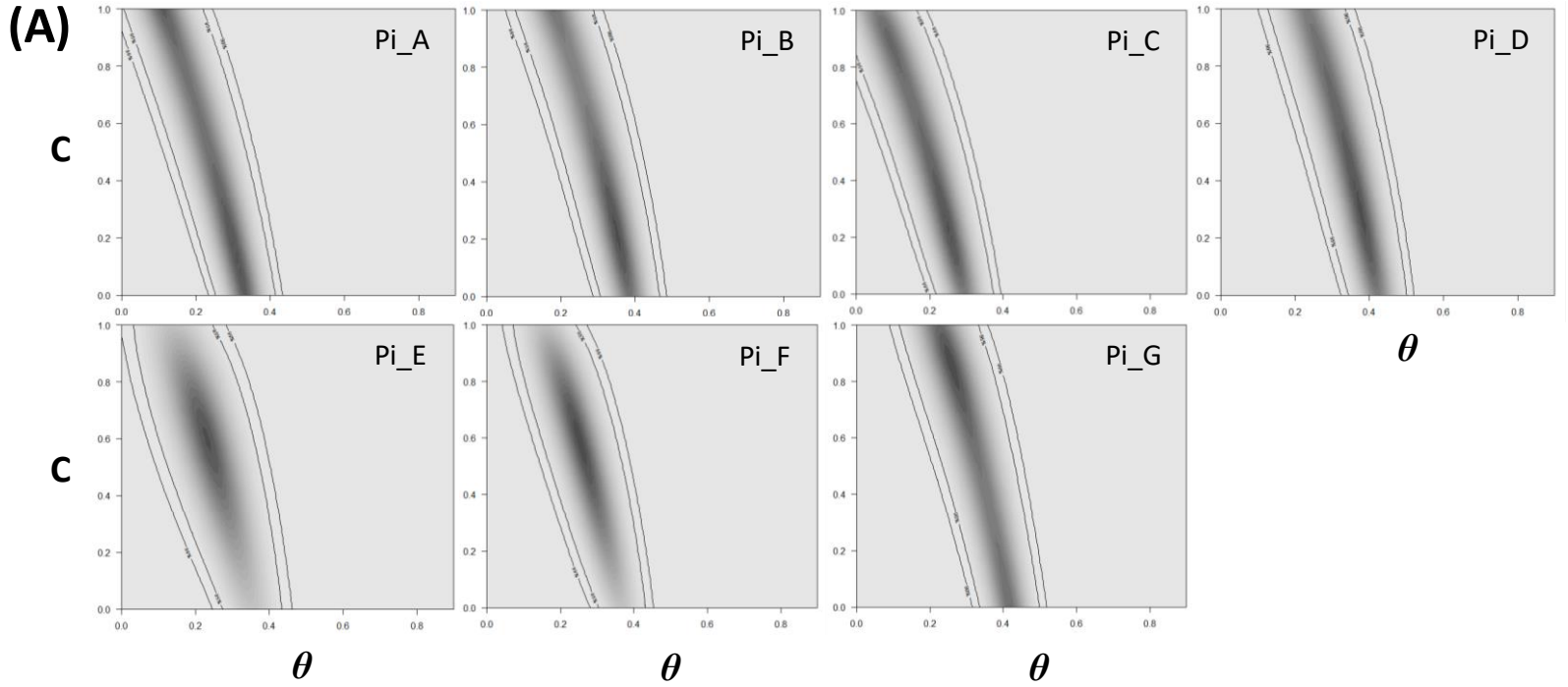

Fig. S1 (continued)

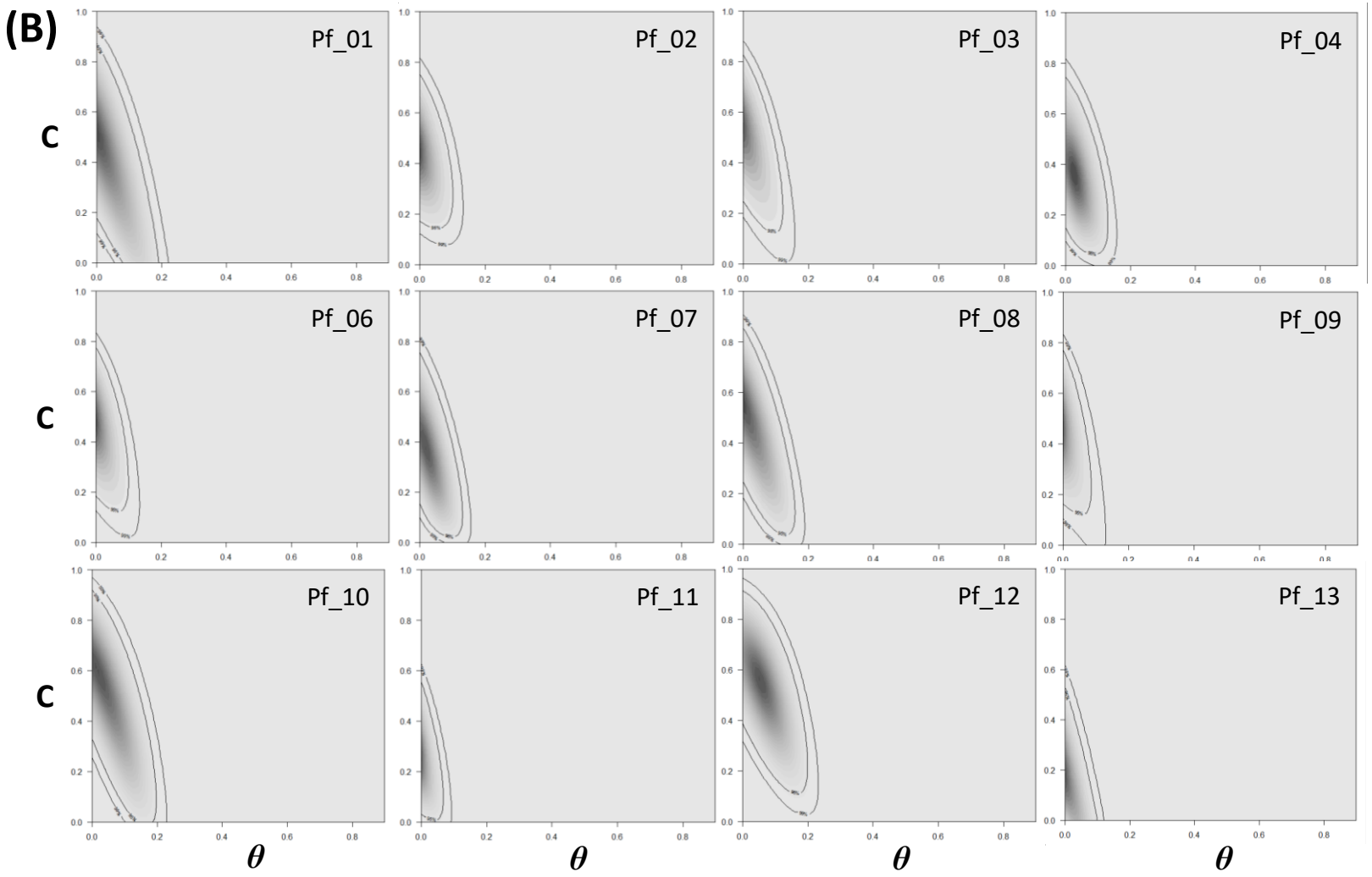

Fig. S1 (continued)

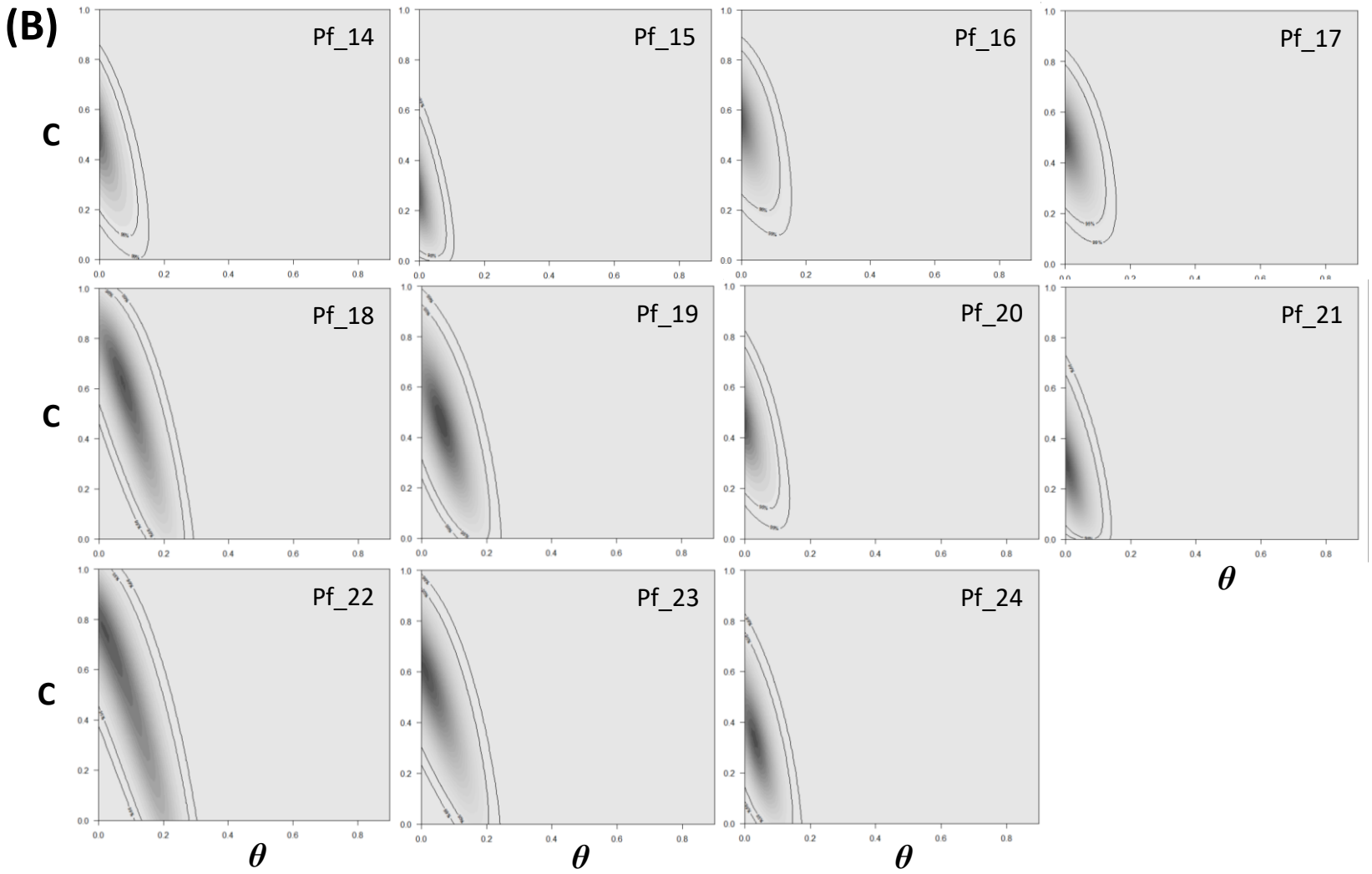

Table S1: Estimation of the variance in family size (S^2_K) from the effective population sizes (N_e) and proportions of inbred matings (C) estimated for each final population. The computation was not possible for Pf_15 (no N_e estimate). The estimation was based on Caballero and Hill (1992, Eq. 10), with α computed from C after Ghai (1969, Eq. 17), and N = 13,200, the census size of initial populations (see Text).

Final populations	C	α	N_e	S^2_K
Pf_01	0.52	0.213	44.59	**721**
Pf_02	0.45	0.170	59.90	**583**
Pf_03	0.54	0.227	41.79	**751**
Pf_04	0.35	0.119	30.95	**1257**
Pf_05	0.00	0.000	24.86	**2122**
Pf_06	0.48	0.188	40.34	**837**
Pf_07	0.38	0.133	112.09	**336**
Pf_08	0.56	0.241	136.54	**223**
Pf_09	0.46	0.176	94.34	**366**
Pf_10	0.59	0.265	193.49	**151**
Pf_11	0.25	0.077	86.50	**494**
Pf_12	0.57	0.249	227.76	**132**
Pf_13	0.18	0.052	136.63	**333**
Pf_14	0.49	0.194	34.48	**968**
Pf_15	0.26	0.081	/	
Pf_16	0.56	0.241	27.06	**1131**
Pf_17	0.50	0.200	28.36	**1163**
Pf_18	0.63	0.299	47.05	**591**
Pf_19	0.46	0.176	58.41	**591**
Pf_20	0.46	0.176	187.01	**184**
Pf_21	0.31	0.101	48.26	**838**
Pf_22	0.75	0.429	56.58	**408**
Pf_23	0.61	0.281	63.56	**450**
Pf_24	0.33	0.110	195.37	**202**

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