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## Research



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# Exploring the causes of small effective population sizes in cyst nematodes using artificial *Globodera pallida* populations

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The effective size of a population is the size of an ideal population which would undergo genetic drift at the same rate as the real population. The balance between selection and genetic drift depends on the 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* and to explore the causes of a low  $N_e/N$  ratio in cyst nematodes using artificial populations. Using a temporal analysis of 24 independent populations, the median  $N_e$  was 58 individuals (min  $N_e = 25$  and max  $N_e = 228$ ).  $N_e$  is commonly lower than  $N$  but in the case of cyst nematodes, the  $N_e/N$  ratio was extremely low. Using artificial populations showed that this low ratio did not result from migration, selection and overlapping generations, but could be explain by the fact that *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  $N_e$ , resulting in a strong intensity of genetic drift, could be important for their control because *G. pallida* populations will have a low capacity to adapt to changing environments.

## 1. 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 smaller than in large populations [1]. When both factors are operating, selection (deterministic) predominates in large populations, while genetic drift (stochastic) predominates in small populations [2–4]. Indeed, within small populations, the random sampling of gametes owing 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 genetic divergence among fragmented populations from the same original source. The balance between the different evolutionary forces (mutation and recombination, selection and migration) and genetic drift depends on the effective population size ( $N_e$ ), rather than the real number of individuals in the population ( $N$ , the census size). The effective size of a population is the size of an ideal population which would undergo genetic drift at the same rate as the observed population [5]. According to the Wright–Fisher model, an ideal population is a diploid species with obligate sexual reproduction and is 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 [1]. Any characteristic of the real population that deviates from the characteristics of the ideal population will cause the effective size ( $N_e$ ) to differ from the census number of individuals in the population ( $N$ ).

Plant pathogens and parasites impose a major constraint on food production worldwide. They are often combated with pesticides, but the need to develop

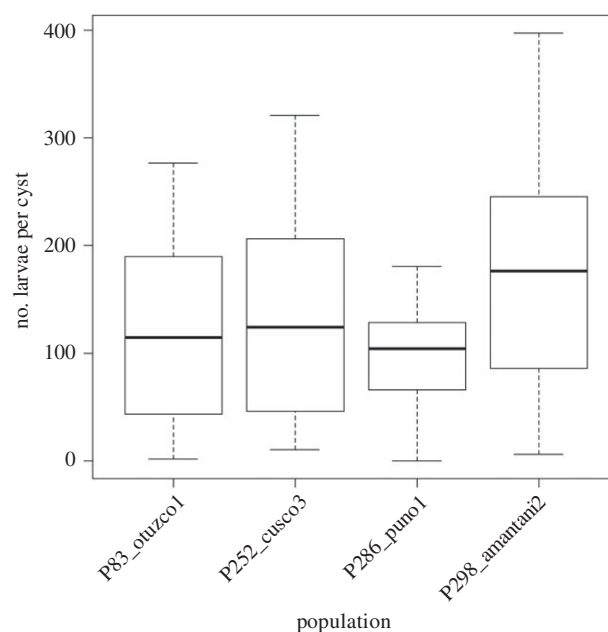
more sustainable production systems fuels a trend towards a limitation of pesticide use. Among possible alternatives, plant resistances look promising, but their durability have to be established. The durability of host resistance is defined as the persistence of resistance efficiency when resistant cultivars are used on large surfaces, over long periods and in the presence of the pathogen [6,7]: durability therefore depends on the pace of adaptive changes of pathogen populations in response to the selection pressure exerted by resistant hosts. The speed of fixation of an advantageous allele depends on its selection coefficient ( $s$ ) but also on the action of genetic drift, which is influenced by the effective population size [8–10]. Consequently, the selection of virulent alleles (the virulence being defined as the ability to infect a resistant host [11–14]) by resistant plants could be partly compromised by low effective population sizes.

Effective population size has been investigated both theoretically [15–18] and measured experimentally [19–21] in a broad variety of organisms. For plant pathogens,  $N_e$ , and thus the importance of genetic drift, has been explored for several plant viruses (e.g. [22–27]) and fungi (e.g. [28–31]), but very scarcely for plant parasitic nematodes (see nevertheless [32]).

Plant-parasitic nematodes cause considerable economic losses in agriculture: worldwide crop losses caused by nematodes have been estimated around US\$100 billion per year [33]. The potato cyst nematode *Globodera pallida* is a quarantine organism regulated in 55 countries [34]. It is a gonochoristic diploid organism with obligate sexual reproduction, which performs only one generation per year under actual European climatic conditions [35]. *Globodera pallida* is probably native to the Andean Cordillera [36], the origin of its unique host genus *Solanum* [37]. This obligate, sedentary endoparasite penetrates the roots as second-stage juveniles (J2) and establishes a syncytium [38], i.e. a particular feeding structure which is a severe nutrient sink for the plant. Sex is environmentally determined and strongly influenced by the size of the syncytium [39]. Adult males leave the root in order to find and mate with females. The females continue to feed and when egg development is finished, they die and form a cyst, enclosing hundreds of eggs, which constitute a survival stage that can remain viable for several years in the soil.

Several methods are available to estimate effective population sizes [20,40,41]. Single-sample methods estimate  $N_e$  from the linkage disequilibrium and/or the heterozygote excess [42–44], whereas temporal methods estimate  $N_e$  from the variation in allelic frequencies between two temporally spaced samples. Deviations from Hardy–Weinberg equilibrium owing to heterozygote deficits have been recorded for three plant parasitic nematode species (*G. pallida* [45], *Heterodera schachtii* [46] and *Globodera tabacum* [47]) and recently attributed to both consanguinity and sub-structure at the within-plant scale [48]. These biological characteristics, inbreeding and sub-structure (Wahlund effect), are known to bias single-sample estimators of  $N_e$  [49–51]. Therefore, temporal methods, such as the one developed by Wang [52], are the most appropriated to estimate  $N_e$  in cyst nematode populations. Because these methods are based on the effect of drift on allele frequency variations,  $N_e$  is called the variance effective size [8,10].

A recent study performed on wild populations of the beet cyst nematode *H. schachtii* showed that the effective population size ( $N_e$ ) and the  $N_e/N$  ratio were very low in cyst nematodes [32]. Rather than working with natural populations, we decided



**Figure 1.** Number of larvae per cyst for the four *G. pallida* Peruvian populations (P83\_otuzco1, P252\_cusco3, P286\_puno1 and P298\_amantani2). No significant difference was observed between those populations ( $F_{3,44} = 1.59$ ;  $p = 0.21$ ).

here to work with artificial populations in order to estimate the  $N_e$  of the potato cyst nematode *G. pallida* and to explore the causes of a low  $N_e/N$  ratio in cyst nematodes. Indeed, using artificial populations allowed us (i) to ensure the absence of migration and of overlapping generations and to reduce and homogenize the action of selection, and thus (ii) to explore the relative contributions of the remaining characteristics which differ from an ideal population (i.e. sex ratio, inbreeding and variance in family size).

## 2. Material and methods

### (a) Initial nematode populations

Twenty-four initial *G. pallida* populations, each composed of 100 cysts, were established by mixing four Peruvian populations that are genetically differentiated and show high allelic richness (P83\_otuzco1, P252\_cusco3, P286\_puno1 and P298\_amantani2 [53]). These *G. pallida* populations are members of the genetic clades I (P286\_puno1 and P298\_amantani2), II (P252\_cusco3) and V (P83\_otuzco1) described by Picard *et al.* [53] and have all been multiplied on the susceptible potato cultivar Désirée. Before mixing these populations, the number of larvae was scored using a magnifying stereomicroscope for 12 randomly chosen cysts which were individually crushed in water, and a one-way ANOVA showed no significant difference in the number of larvae per cyst between those four populations ( $F_{3,44} = 1.59$ ;  $p = 0.21$ ; figure 1). The initial census size was thus estimated by multiplying the number of cysts (i.e. 100) by the mean number of larvae per cyst (i.e. 132, figure 1).

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, except for the equal mix, which was replicated six times, for a total of 24 initial populations (electronic supplementary material, table S1). Seven initial populations (Pi\_A to Pi\_G), composed of 50 cysts, were also prepared in the same proportions (i.e. one population for each proportion) for the estimation of initial allelic frequencies.

## (b) 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 a virulence allele. Moreover, because plants propagated vegetatively from tubers are clones, 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 one plant and selecting against it in another plant).

For each initial population, the 100 cysts were locked in a tulle bag and placed in a 13 cm pot three-quarter 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 16 h 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.

## (c) Microsatellite genotyping

The 31 *G. pallida* populations (i.e. seven initial and 24 final populations) were genotyped using 12 microsatellite markers (Gp106, Gp108, Gp109, Gp111, Gp112, Gp116, Gp117, Gp118, Gp122, Gp126, Gp135 and Gp145) developed by Montarry *et al.* [48] directly from the *G. pallida* genome [54]. For each population, from 26 (for Pi\_E) to 40 (for Pi\_C) larvae, coming from distinct and randomly chosen cysts, were successfully genotyped. Two multiplex panels were used to genotype the 1105 individuals at the 12 loci.

DNA from a single larva (i.e. one second-stage juvenile J2) was extracted following a procedure using sodium hydroxide and proteinase K [55]. Polymerase chain reaction (PCR) was performed using a 384-well reaction module (BIO-RAD C1000) in a 5 µl volume containing 1X of Type-it Microsatellite PCR kit (QIAGEN), 0.4 µM of primer mix and 1 µl of template DNA. Cycling conditions included an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 90 s and extension at 72°C for 30 s, followed by a final extension at 60°C for 30 min. PCR products were then diluted to 1:25 in sterile water and 3 µl of this dilution were mixed with 0.05 µl of GeneScan 500 LIZ Size Standard (Applied Biosystems) and 5 µl of formamide (Applied Biosystems). Analyses of PCR products were conducted on ABI Prism® 3130xl sequencer (Applied Biosystems). Allele sizes were determined by the automatic calling and binning module of GENE MAPPER v. 4.1 (Applied Biosystems) with manual examination of irregular results. To minimize the rate of genotyping errors, a second round of PCR and electrophoresis was performed for 10% of the global number of individuals.

## (d) Population genetic characteristics

Genetic diversity of each nematode population was estimated through allelic richness ( $A_r$ ) and unbiased gene diversity ( $H_{nb}$ ) [56]. Departure from Hardy–Weinberg equilibrium was tested through the  $F_{IS}$  estimation for each population.  $H_{nb}$  and  $F_{IS}$  were computed using GENETIX 4.05.2 [57]. The statistical significance of  $F_{IS}$  values for each population was tested using the allelic permutation method (1000 permutations) implemented in GENETIX.  $A_r$  was estimated on a reduced sample of 26 individuals using the rarefaction method implemented in populations 1.2.32 [58]. We compared gene diversity (both  $H_{nb}$  and  $A_r$ ) between initial and final populations by means of two-sided

permutation tests for paired data (10 000 permutations; R-code available upon request from the authors).

Because heterozygote deficits in cyst nematodes could be owing to a Wahlund effect (i.e. sub-structure) and/or to consanguinity [48], we used the method of Overall & Nichols [59] in order to calculate a likelihood surface for the genetic correlation owing to population subdivision ( $\theta$ ) and the proportion of the population practising 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 assuming a degree of relatedness of 1/4 (see [48]) to all initial and final populations showing significant heterozygote deficits. Likelihood estimates were obtained by searching for the maximum of the likelihood function over a grid of 10 000 combinations of  $\theta$  and  $C$  values, and graphs of the likelihood surface were obtained for each nematode population using the statistical software R version 3.1.1 [60].

## (e) Effective population size estimation

The temporal method developed by Wang [52] was used to estimate  $N_e$  for the 24 independent pairs of initial and final populations of *G. pallida*. This likelihood-based method is implemented in the MLNE 1.0 software [61].

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 coefficient. All statistical analyses were performed using R.

## (f) The causes of a low $N_e/N$ ratio in cyst nematodes

Sweepstake reproduction, which is linked to high fecundities, and hence common to many marine and parasitic species, has been put forward as an explanation for highly reduced  $N_e/N$  ratios [62–64]. This has motivated the development of alternatives to the Wright–Fisher model [65], or to its coalescent counterpart, the Kingman coalescent (see [63,64]). Williamson & Slatkin [66] have indicated that maximum-likelihood methods could lead to the simultaneous estimation of variance effective size and variance in family size, but we are not aware that any such model has been developed yet. Multi-merger coalescents have been designed specifically to take sweepstake reproduction into account [64] but current applications rely on the infinite-many-site models and are thus restricted to the analysis of sequence data [67,68]. Consequently, we estimated the variance in family sizes using the corrected equation proposed by Caballero & Hill [69], which is a modified version of the Wright–Fisher model that takes into account bi-parental inbreeding (see also [65]):

$$S_k^2 = \frac{4N - 2N_e(1 - \alpha)}{N_e(1 + 3\alpha)},$$

where  $S_k^2$  is the variance of family size,  $N$  is the census size,  $N_e$  is the variance effective population size and  $\alpha$  is the departure from Hardy–Weinberg proportions. In the present case,  $N$  was fixed to the census size of initial populations (i.e. 13 200) and the effective population sizes and the proportions of inbred mating estimated for each final population were used. The  $\alpha$  parameter was computed from  $C$ , the proportion of the population practising consanguinity (estimated using the method of Overall & Nichols [59]), according to Ghai [70]:

$$\alpha = \frac{C}{4 - 3C}.$$

**Table 1.** 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 italics. For each population showing a significant heterozygote deficit,  $\theta$  and  $C$  values corresponding to the maximum-likelihood were indicated.)

| population          | cysts | $n$ | $H_{nb}$ | $A_r$ | $F_{IS}$    | $\theta$ | $C$  |
|---------------------|-------|-----|----------|-------|-------------|----------|------|
| initial populations |       |     |          |       |             |          |      |
| Pi_A                | 100   | 39  | 0.68     | 7.23  | <i>0.37</i> | 0.31     | 0.13 |
| Pi_B                | 100   | 38  | 0.68     | 7.23  | <i>0.41</i> | 0.35     | 0.23 |
| Pi_C                | 100   | 40  | 0.65     | 7.34  | <i>0.32</i> | 0.25     | 0.24 |
| Pi_D                | 100   | 39  | 0.64     | 6.25  | <i>0.43</i> | 0.38     | 0.28 |
| Pi_E                | 100   | 26  | 0.65     | 6.57  | <i>0.37</i> | 0.23     | 0.60 |
| Pi_F                | 100   | 38  | 0.69     | 7.30  | <i>0.39</i> | 0.25     | 0.60 |
| Pi_G                | 100   | 36  | 0.68     | 6.90  | <i>0.43</i> | 0.26     | 0.85 |
| final populations   |       |     |          |       |             |          |      |
| Pf_01               | 2704  | 28  | 0.65     | 7.00  | <i>0.14</i> | 0.00     | 0.52 |
| Pf_02               | 3407  | 33  | 0.66     | 6.51  | <i>0.14</i> | 0.00     | 0.45 |
| Pf_03               | 3136  | 38  | 0.68     | 6.31  | <i>0.16</i> | 0.00     | 0.54 |
| Pf_04               | 2227  | 36  | 0.59     | 6.35  | <i>0.11</i> | 0.03     | 0.35 |
| Pf_05               | 2541  | 34  | 0.60     | 5.54  | −0.01       | .        | .    |
| Pf_06               | 2082  | 36  | 0.62     | 6.24  | <i>0.13</i> | 0.00     | 0.48 |
| Pf_07               | 2053  | 37  | 0.67     | 7.14  | <i>0.10</i> | 0.02     | 0.38 |
| Pf_08               | 2511  | 38  | 0.70     | 6.87  | <i>0.17</i> | 0.00     | 0.56 |
| Pf_09               | 1459  | 32  | 0.65     | 6.59  | <i>0.12</i> | 0.00     | 0.46 |
| Pf_10               | 2193  | 34  | 0.66     | 6.78  | <i>0.18</i> | 0.02     | 0.59 |
| Pf_11               | 2425  | 37  | 0.63     | 6.27  | <i>0.05</i> | 0.00     | 0.25 |
| Pf_12               | 1749  | 39  | 0.64     | 6.40  | <i>0.22</i> | 0.05     | 0.57 |
| Pf_13               | 2893  | 33  | 0.63     | 6.29  | <i>0.05</i> | 0.00     | 0.18 |
| Pf_14               | 1391  | 36  | 0.61     | 6.45  | <i>0.13</i> | 0.00     | 0.49 |
| Pf_15               | 1060  | 35  | 0.68     | 6.77  | <i>0.07</i> | 0.00     | 0.26 |
| Pf_16               | 2613  | 36  | 0.60     | 6.00  | <i>0.18</i> | 0.00     | 0.56 |
| Pf_17               | 2161  | 36  | 0.60     | 5.93  | <i>0.16</i> | 0.00     | 0.50 |
| Pf_18               | 1641  | 39  | 0.63     | 6.82  | <i>0.22</i> | 0.07     | 0.63 |
| Pf_19               | 2776  | 34  | 0.60     | 6.37  | <i>0.17</i> | 0.06     | 0.46 |
| Pf_20               | 2117  | 37  | 0.65     | 6.73  | <i>0.13</i> | 0.00     | 0.46 |
| Pf_21               | 1753  | 35  | 0.61     | 6.19  | <i>0.08</i> | 0.00     | 0.31 |
| Pf_22               | 1815  | 34  | 0.62     | 6.53  | <i>0.21</i> | 0.02     | 0.75 |
| Pf_23               | 2056  | 36  | 0.61     | 6.69  | <i>0.18</i> | 0.01     | 0.61 |
| Pf_24               | 1779  | 36  | 0.64     | 6.81  | <i>0.11</i> | 0.03     | 0.33 |

### 3. Results

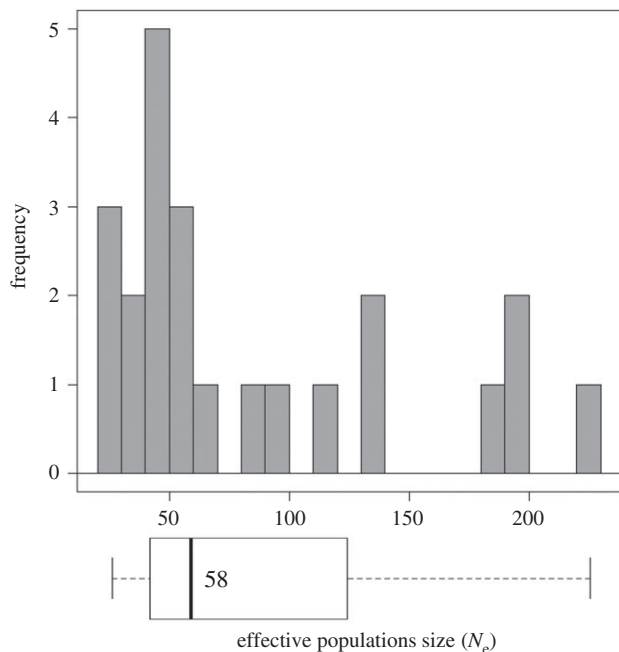
#### (a) Genetic characteristics of initial and final populations

As expected, genetic diversity was high for all initial populations ( $0.64 < H_{nb} < 0.69$  and  $6.25 < A_r < 7.34$ ; table 1). This diversity, though still high in final populations ( $0.59 < H_{nb} < 0.70$  and  $5.54 < A_r < 7.14$ ; table 1), decreased from one generation to the next ( $p = 0.0008$  for  $H_{nb}$  and  $p = 0.0003$  for  $A_r$ ). 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 1). The outputs of the method of Overall & Nichols

[59] showed that heterozygote deficits were owing to consanguinity and substructure for the seven initial populations (table 1 and electronic supplementary material, figure S1-A) and only to consanguinity for the 23 final populations showing significant heterozygote deficits (table 1 and electronic supplementary material, figure S1-B).

#### (b) 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 (figure 2). Among the 24 pairs of initial and final populations, only one pair (Pi\_15 and Pf\_15) showed an infinite  $N_e$ ,



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

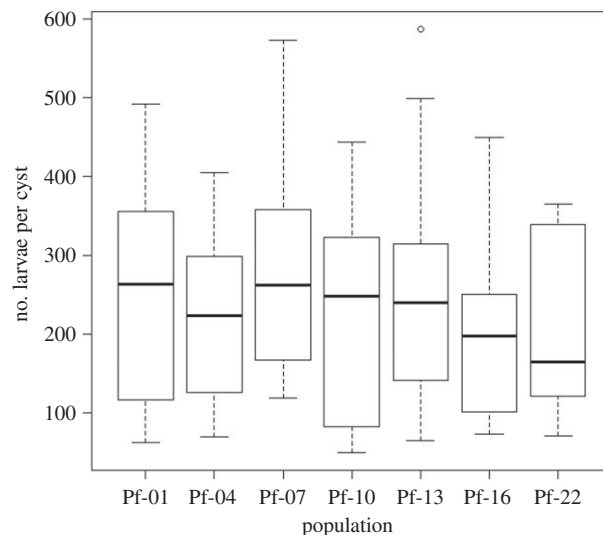
indicating a small variation in allele frequencies between the initial population and the final one.

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 homogeneous 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 1060 to 3407 with a mean ( $\pm$  s.e.m.) of 2189 ( $\pm$  116). There was no correlation between  $N_e$  estimates and the number of newly formed cysts (Pearson's coefficient  $\text{cor} = -0.18$ ;  $p = 0.41$ ). The number of larvae per cyst, scored for seven final populations, ranged from 196 to 288 with a mean ( $\pm$  s.e.m.) of 235 ( $\pm$  12), and a one-way ANOVA showed no significant difference for the number of larvae per cyst among these seven final populations ( $F_{6,77} = 0.74$ ;  $p = 0.62$ ; figure 3). Consequently, our estimation of the mean final census size ( $N$ ) across all populations was 514 415 larvae (2189 newly formed cysts \* 235 larvae per cyst). The higher number of larvae per cyst in the final than in the initial populations could be owing to the fact that the final populations correspond to newly formed cysts, whereas the initial populations have been stored at 4°C before the estimation of the number of larvae per cyst.

### (c) The causes of a low $N_e/N$ ratio in cyst nematodes

Some characteristics of our artificial *G. pallida* populations are similar to an ideal population, i.e. no migration, no selection, no possibility for variation of  $N$  over generations and no overlapping generations. However, those populations deviate from the assumptions of the ideal population by having unequal sex ratios and showing non-random union of gametes. These factors on their own are however not able to explain a very



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

low  $N_e/N$  ratio [10], observed here in *G. pallida* and previously in the beet cyst nematode *H. schachtii* [32].

We have thus estimated how our artificial *G. pallida* populations deviate from an ideal population in term of variance in family sizes, the assumption of the classic Wright–Fisher model being a Poisson distribution of family sizes. Using our experimental data, we estimated the variance in family sizes ( $S_f^2$ ) to range between 130 and 2100 (table 2), suggesting an unequal success of parents in producing progeny [63].

## 4. Discussion

This report evaluates the effective size of populations of *G. pallida*, the potato cyst nematode. Rather than working with natural populations, which could sometimes harbour very low genetic diversity, 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 owing 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 owing to consanguinity, as in natural *G. pallida* populations [48]. This result suggests that mating patterns are similar in our artificial populations and in natural field populations. Moreover, temporal methods assume neither migration nor selection and that the variation in allele frequencies between the samples is only owing to genetic drift. Working with artificial populations is a way to ensure the absence of migration, and the use of a susceptible potato cultivar reduces the action of selection. 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 the infection cycle of plant virus populations is quite complicated because of the lack of estimates of generation times for viruses [27]. Regarding the beet cyst nematode *H. schachtii*, which is a plurivoltine species, Jan *et al.* [32] have used two extreme estimations of the generation number. We have not had that problem using the monovoltine species *G. pallida* which performed only one generation over the experiment.

**Table 2.** Estimation of the variance in family size ( $S_k^2$ ) from the effective population sizes ( $N_e$ ) and the departure from Hardy–Weinberg proportions ( $\alpha$ ) computed from proportions of inbred matings ( $C$ ) estimated for each final population. (The computation was not possible for Pf\_15 (no  $N_e$  estimate).)

| final populations | $C$  | $\alpha$ | $N_e$  | $S_k^2$ |
|-------------------|------|----------|--------|---------|
| 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     |

Using the likelihood-based method developed by Wang [52], the median of the 24  $N_e$  estimates was 58 individuals. Consistent with these low effective population sizes, we observed a decrease in both allelic richness and expected heterozygosity over a single generation. 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 (2189 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 [71], yielding  $\bar{N} = 25\,740$ , and thus  $N_e/N \approx 2.10^{-3}$ . Based on a meta-analysis, values of  $N_e/N$  average only 10–15% [40,72]. 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 2000 adults, but its effective size was estimated to be only 85 ( $N_e/N = 0.04$  [73]).  $N_e$  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 (e.g. [74]). 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 of less than 1% [32]. 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* [75–77] and of animal parasitic nematodes (e.g. for *Trichostrongylus axei* [78]).

The effective size of a population is the size of an ideal population which would undergo genetic drift at the same rate as the observed population [5] 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% [79]. Because the  $N_e/N$  ratio and the sex ratio (SR) are related through the relation  $N_e/N = 4 * SR * (1 - SR)$ , a 60% sex ratio would lead to a 4% reduction in the  $N_e/N$  ratio when compared to a balanced sex ratio. A meta-analysis showed that unequal sex ratios reduce effective population sizes below actual sizes by about 36% [72]. Biased sex ratios are thus unlikely to explain our results. *Globodera pallida* populations are characterized by high levels of inbreeding, highlighted here for artificial populations (i.e.  $F_{IS}$  significantly higher than zero owing to consanguinity) and previously highlighted for natural populations [48], which could also reduce effective population sizes [10]. While random mating generally sustains effective population sizes of pathogens [80], inbreeding increases the extent of genetic drift in some pathogen populations, resulting in reduced  $N_e$  [81]. 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 [10]. 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 a population expansion and thus that on average more than one offspring was 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 [82,83]. Patterns of mitochondrial gene diversity between larvae from the same cyst support the same mating pattern for *G. pallida* (J. Ferreira de Carvalho, S. Fournet and E. J. Petit 2009, unpublished). Our estimation of the variance in family sizes, which takes into account bi-parental inbreeding, ranged between 130 and 2100, suggesting an unequal success of parents in producing progeny [63]. 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  $2 * 2189 / 13\,200 = 0.33$ , meaning that at least 2/3 of all individuals have zero mating 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 eqn 5c in [63]). Ultimately, it is the combined impact of the

three factors (i.e. high variance in family sizes, unequal sex ratios, and inbreeding) which could explain why the  $N_e/N$  ratio is extremely low in *G. pallida* populations. We here estimated effective sizes from the temporal variation in allele frequencies to then explore potential causes of low  $N_e/N$  ratios. More accurate predictions of effective sizes for this system would require us to independently estimate all variance and covariance components of the reproductive success in this species to be able to fill all terms of a model that is able to consider bi-parental inbreeding, unbalanced sex ratios and variance in family size together (eqn 28 in [84]). Our artificial populations differ from field populations of cyst nematodes in some respects. The sex ratio could be more unbalanced in the field than in our artificial populations because most of the resistant potato cultivars masculinize nematode populations. Similarly, the variance in family sizes could be higher in field populations than in artificial ones because field populations are composed not only of newly formed cysts but also of older cysts, as nematode's cysts are able to survive several years in the soil. Because field populations may have more unbalanced sex ratios and greater variance in reproductive output than our artificial populations, the results we obtained from our experiments are likely to represent best-case scenarios as compared to field conditions.

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 strong and selection highly alters the genetic composition of populations, whereas, in populations with a small  $N_e$ , genetic drift is important and counters the effect of selection. Exploring the relationship between the probability of fixation of an allele in a population and its selective advantage (i.e. the selection coefficient) under a Wright–Fisher model [85] showed that considering the effective population size provides contrasting probabilities of fixation of an advantageous allele, whereas with the census size, this probability is very strong whatever the strength of selection (electronic supplementary material, figure S2). However, Der *et al.* [86] showed using the Eldon–Wakeley model [87] that selection operates very differently for species with skewed offspring numbers (i.e. with a high variance in

family size). Their work demonstrates that, for the same selection pressure and the same  $N_e$ , an advantageous allele has a higher probability of fixation in populations with skewed than with Poisson-distributed offspring numbers, even at very low  $N_e$ . Consequently, despite the presence of genetic drift, the adaptation of cyst nematodes to plant resistances, i.e. the fixation of the virulence alleles, will be possible, as shown for *G. pallida* by previous results from experimental evolution on different resistant potato genotypes [79,88,89]. As in the Wright–Fisher model, though, the fate of an advantageous allele depends on the product of the selection coefficient and of the effective size. This reinforces the idea that durable strategies of resistance deployment should favour the ones that will enable the action of drift, such as the use of resistant cultivars in rotation with susceptible ones. In addition, such strategies should consider that in natural field populations of *G. pallida*, gene flow [45] could partly compensate the impact of genetic drift [40]. In cyst nematodes, gene flow has mainly been attributed to the passive transport of cysts through agricultural practices [47]. Therefore, all agricultural management strategies that reduce gene flow and thus promote small effective population sizes would be beneficial for the durability of plant resistance.

**Data accessibility.** A file (Ne\_G\_pallida.txt) containing the genotypic data (Genepop format) for each initial (Pi\_A to Pi\_G) and final populations (Pf-01 to Pf-24) is available from the Dryad Digital Repository at: <http://dx.doi.org/10.5061/dryad.7t3j55p> [90].

**Authors' contributions.** S.B.-V., R.M. and J.M. performed the experiments according to a protocol elaborated jointly by S.F., E.G. and J.M. P.-L.J., E.J.P. and J.M. analysed the data. S.F., E.J.P., E.G. and J.M. wrote the text and prepared the figures. All authors gave final approval for publication.

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