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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 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).

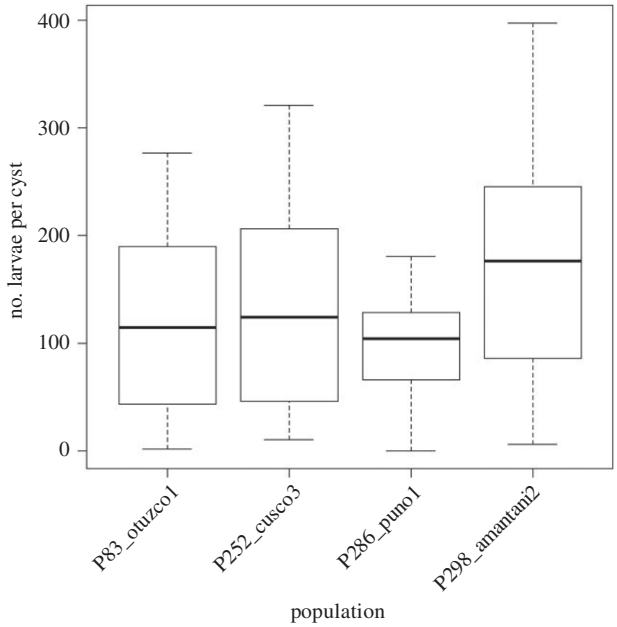

Figure 1. Number of larvae per cyst for the four *G. pallida* Peruvian populations (P83_otuzco1, P252_cusco3, P286_puno1 and P298_amantani2). No significant difference was observed between those populations ($F_{3,44} = 1.59$; $p = 0.21$).

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 GeneMapper 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 (θ) 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 where obtained by searching for the maximum of the likelihood function over a grid of 10 000 combinations of θ 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 α 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 α 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, 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

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,

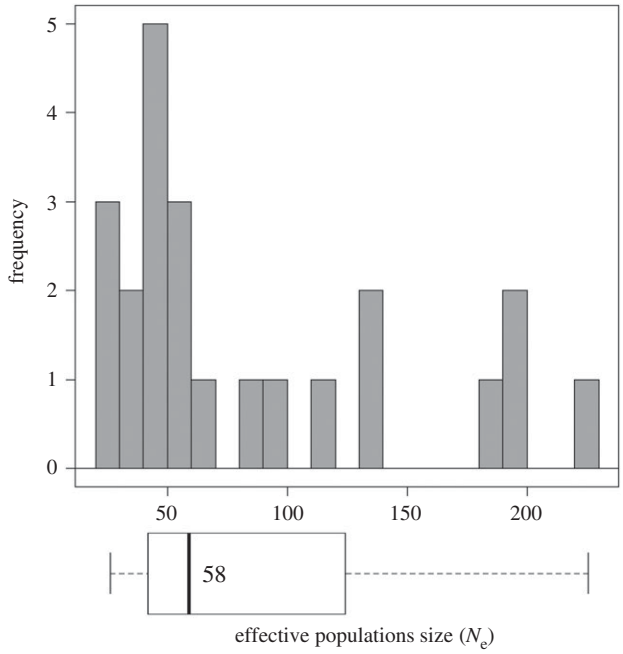

Figure 2. Histogram showing the distribution of the independent effective population sizes estimated using Wang's method. The median N_e is indicated directly onto the box plot below the histogram.

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 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

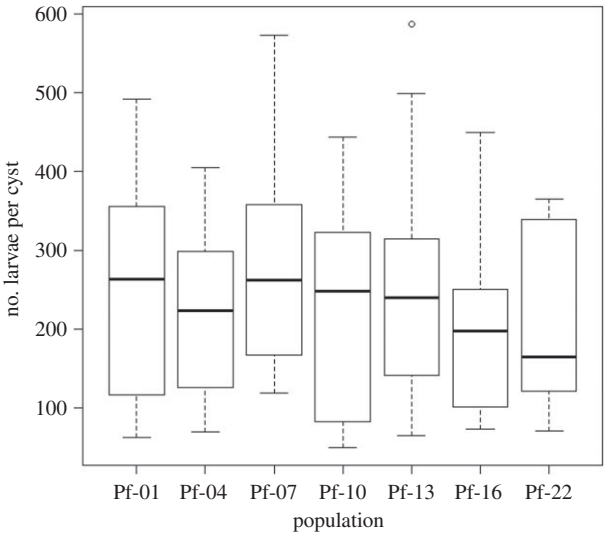

Figure 3. Number of larvae per cyst for the seven final *G. pallida* populations. No significant difference was observed between those populations ($F_{6,77} = 0.74$; $p = 0.62$).

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_k^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 (α) 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	α	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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References

1. Frankham R, Ballou JD, Briscoe DA. 2002 *Introduction to conservation genetics*. Cambridge, UK: Cambridge University Press.
2. Kimura M, Maruyama T, Crow JF. 1963 The mutation load in small populations. *Genetics* **48**, 1303–1312.
3. Nei M, Maruyama T, Chakaraborty R. 1975 The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10. (doi:10.2307/2407137)
4. Gherman A *et al*. 2007 Population bottlenecks as a potential major shaping force of human genome architecture. *PLoS Genet.* **3**, 1223–1231. (doi:10.1371/journal.pgen.0030119)
5. Wright S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97–159.
6. Johnson R. 1981 Durable resistance: definition of genetic control and attainment in plant breeding. *Phytopathology* **71**, 567–568. (doi:10.1094/Phyto-71-567)
7. Johnson R. 1984 A critical analysis of durable resistance. *Annu. Rev. Phytopathol.* **22**, 309–330. (doi:10.1146/annurev.py.22.090184.001521)
8. Crow JF, Kimura M. 1970 *An introduction to population genetics theory*. New York: NY: Harper and Row.
9. Fraser AS. 1972 An introduction to population genetic theory. In *Teratology*, vol. 5 (eds JF Crow, M Kimura), pp. 386–387. New York: NY: Harper and Row.
10. Charlesworth B. 2009 Effective population size and patterns of molecular evolution and variation. *Nat. Rev. Genet.* **10**, 195–205. (doi:10.1038/nrg2526)
11. Vanderplank JE. 1963 *Disease resistance in plants*. New York: NY: Academic Press.
12. Gandon S, Michalakis Y. 2002 Local adaptation, evolutionary potential and host–parasite coevolution: interactions between migration, mutation, population size and generation time. *J. Evol. Biol.* **15**, 451–462. (doi:10.1046/j.1420-9101.2002.00402.x)
13. Tellier A, Brown JKM. 2007 Stability of genetic polymorphism in host-parasite interactions. *Proc. R. Soc. B* **274**, 809–817. (doi:10.1098/rspb.2006.0281)
14. Tellier A, Brown JKM. 2009 The influence of perenniality and seed banks on polymorphism in plant-parasite interactions. *Am. Nat.* **174**, 769–779. (doi:10.1086/646603)
15. Crow JF, Kimura M. 1972 The effective number of a population with overlapping generations: a correction and further discussion. *Am. J. Hum. Genet.* **24**, 1–10.
16. Nei M, Tajima F. 1981 Genetic drift and estimation of effective population size. *Genetics* **98**, 625–640.

17. Tajima F, Nei M. 1984 Note on genetic drift and estimation of effective population size. *Genetics* **106**, 569–574.
18. Criscione CD, Blouin MS. 2005 Effective sizes of macroparasite populations: a conceptual model. *Trends Parasitol.* **21**, 212–217. (doi:10.1016/j.pt.2005.03.002)
19. Johnson JA, Bellinger MR, Toepfer JE, Dunn P. 2004 Temporal changes in allele frequencies and low effective population size in greater prairie-chickens. *Mol. Ecol.* **13**, 2617–2630. (doi:10.1111/j.1365-294X.2004.02264.x)
20. Wang J. 2005 Estimation of effective population sizes from data on genetic markers. *Phil. Trans. R. Soc. B* **360**, 1395–1409. (doi:10.1098/rstb.2005.1682)
21. Araki H, Waples RS, Blouin MS. 2007 A potential bias in the temporal method for estimating Ne in admixed populations under natural selection. *Mol. Ecol.* **16**, 2261–2271. (doi:10.1111/j.1365-294X.2007.03307.x)
22. Betancourt M, Fereres A, Fraile A, García-Arenal F. 2008 Estimation of the effective number of founders that initiate an infection after aphid transmission of a multipartite plant virus. *J. Virol.* **82**, 12 416–12 421. (doi:10.1128/JVI.01542-08)
23. Monsion B, Froissart R, Michalakis Y, Blanc S. 2008 Large bottleneck size in cauliflower mosaic virus populations during host plant colonization. *PLoS Pathog.* **4**, e1000174. (doi:10.1371/journal.ppat.1000174)
24. Zwart MP, Daròs JA, Elena SF. 2011 One is enough: *in vivo* effective population size is dose-dependent for a plant RNA virus. *PLoS Pathog.* **7**, e1002122. (doi:10.1371/journal.ppat.1002122)
25. Gutiérrez S, Michalakis Y, Blanc S. 2012 Virus population bottlenecks during within-host progression and host-to-host transmission. *Curr. Opin. Virol.* **2**, 1–10. (doi:10.1016/j.coviro.2012.08.001)
26. Fabre F, Montarry J, Coville J, Senoussi R, Simon V, Moury B. 2012 Modelling the evolutionary dynamics of viruses within their hosts: a case study using high-throughput sequencing. *PLoS Pathog.* **8**, e1002654. (doi:10.1371/journal.ppat.1002654)
27. Fabre F, Moury B, Johansen EI, Simon V, Jacquemond M, Senoussi R. 2014 Narrow bottlenecks affect pea seedborne mosaic virus populations during vertical transmission but not during leaf colonization. *PLoS Pathog.* **10**, e1003833. (doi:10.1371/journal.ppat.1003833)
28. Damgaard C, Giese H. 1996 Genetic variation in Danish populations of *Erysiphe graminis* f.sp. *hordei*: estimation of gene diversity and effective population size using RFLP data. *Plant Pathol.* **45**, 691–696. (doi:10.1046/j.1365-3059.1996.d01-165.x)
29. Zhan J, Mundt CC, McDonald BA. 2001 Using restriction fragment length polymorphisms to assess temporal variation and estimate the number of ascospores that initiate epidemics in field populations of *Mycosphaerella graminicola*. *Phytopathology* **91**, 1011–1017. (doi:10.1094/PHYTO.2001.91.10.1011)
30. Duan X, Tellier A, Wan A, Leconte M, de Vallavielle-Pope C, Enjalbert J. 2010 *Puccinia striiformis* f.sp *tritici* presents high diversity and recombination in the over-summering zone of Gansu, China. *Mycologia* **102**, 44–53. (doi:10.3852/08-098)
31. Stukenbrock EH, Bataillon T, Dutheil JY, Hansen TT, Li R, Zala M, McDonald BA, Wang J, Schierup MH. 2011 The making of a new pathogen: insights from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species. *Genome Res.* **21**, 2157–2166. (doi:10.1101/gr.118851.110)
32. Jan PL, Gracianne C, Fournet S, Olivier E, Arnaud JF, Porte C, Bardou-Valette S, Denis MC, Petit EJ. 2016 Temporal sampling helps unravel the genetic structure of naturally occurring populations of a phytoparasitic nematode. 1. Insights from the estimation of effective population sizes. *Evol. Appl.* **9**, 489–501. (doi:10.1111/eva.12352)
33. Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland S, Maafi ZT. 2011 Current nematode threats to world agriculture. In *Genomics and molecular genetics of plant-nematode interactions* (eds J Jones, G Gheysen, C Fenoll), pp. 21–43. Dordrecht, The Netherlands: Springer.
34. Gamel S, Letort A, Fouville D, Folcher L, Grenier E. 2017 Development and validation of real-time PCR assays based on novel molecular markers for the simultaneous detection and identification of *Globodera pallida*, *G. rostochiensis* and *Heterodera schachtii*. *Nematology* **19**, 789–804. (doi:10.1163/15685411-00003086)
35. Ebrahimi N, Viaene N, Demeulemeester K, Moens M. 2014 Observations on the life cycle of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, on early potato cultivars. *Nematology* **16**, 937–952. (doi:10.1163/15685411-00002821)
36. Grenier E, Fournet S, Petit E, Anthoine G. 2010 A cyst nematode 'species factory' called the Andes. *Nematology* **12**, 163–169. (doi:10.1163/138855409X12573393054942)
37. Hijmans RJ, Spooner DM. 2001 Geographic distribution of wild potato species. *Am. J. Bot.* **88**, 2101–2112. (doi:10.2307/3558435)
38. Jones MGK, Northcote DH. 1972 Nematode-induced syncytium: a multinucleate transfer cell. *J. Cell Sci.* **10**, 789–809.
39. Sobczak M, Golinowski W. 2011 Cyst nematodes and syncytia. In *Genomics and molecular genetics of plant-nematode interactions* (eds JT Jones, G Gheysen, C Fenoil), pp 61–82. Dordrecht, The Netherlands: Springer.
40. Palstra FP, Ruzzante DE. 2008 Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* **17**, 3428–3447. (doi:10.1111/j.1365-294X.2008.03842.x)
41. Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010 Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv. Genet.* **11**, 355–373. (doi:10.1007/s10592-010-0050-7)
42. Pudovkin AI, Zaykin DV, Hedgecock D. 1996 On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics* **144**, 383–387.
43. Tallmon DA, Koyuk A, Luikart G, Beaumont MA. 2008 Onesamp: a program to estimate effective population size using approximate Bayesian computation. *Mol. Ecol. Resour.* **8**, 299–301. (doi:10.1111/j.1471-8286.2007.01997.x)
44. Waples RS, Do CHI. 2008 LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Resour.* **8**, 753–756. (doi:10.1111/j.1755-0998.2007.02061.x)
45. Picard D, Plantard O, Scurrah M, Mugniéry D. 2004 Inbreeding and population structure of the potato cyst nematode (*Globodera pallida*) in its native area (Peru). *Mol. Ecol.* **13**, 2899–2908. (doi:10.1111/j.1365-294X.2004.02275.x)
46. Plantard O, Porte C. 2004 Population genetic structure of the sugar beet cyst nematode *Heterodera schachtii*: a gonochoristic and amphimictic species with highly inbred but weakly differentiated populations. *Mol. Ecol.* **13**, 33–41. (doi:10.1046/j.1365-294X.2003.02023.x)
47. Alenda C, Montarry J, Grenier E. 2014 Human influence on the dispersal and genetic structure of French *Globodera tabacum* populations. *Infect. Genet. Evol.* **27**, 309–317. (doi:10.1016/j.meegid.2014.07.027)
48. Montarry J, Jan PL, Gracianne C, Overall ADJ, Bardou-Valette S, Olivier E, Fournet S, Grenier E, Petit EJ. 2015 Heterozygote deficits in cyst plant-parasitic nematodes: possible causes and consequences. *Mol. Ecol.* **24**, 1654–1667. (doi:10.1111/mec.13142)
49. Zhdanova OL, Pudovkin AI. 2008 Nb_HetEx: a program to estimate the effective number of breeders. *J. Hered.* **99**, 694–695. (doi:10.1093/jhered/esn061)
50. Waples RS, Do CHI. 2010 Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: an untapped resource for applied conservation and evolution. *Evol. Appl.* **3**, 244–262. (doi:10.1111/j.1752-4571.2009.00104.x)
51. Holleley CE, Nichols RA, Whitehead MR, Adamack AT, Gunn MR, Sherwin WB. 2014 Testing single-sample estimators of effective population size in genetically structured populations. *Conserv. Genet.* **15**, 23–35. (doi:10.1007/s10592-013-0518-3)
52. Wang J. 2001 A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genet. Res.* **78**, 243–257. (doi:10.1017/S0016672301005386)
53. Picard D, Sempere T, Plantard O. 2007 A northward colonisation of the Andes by the potato cyst nematode during geological times suggests multiple host-shifts from wild to cultivated potatoes. *Mol. Phylogenet. Evol.* **42**, 308–316. (doi:10.1016/j.ympev.2006.06.018)
54. Cotton JA *et al.* 2014 The genome and life-stage specific transcriptomes of *Globodera pallida*

elucidate key aspects of plant parasitism by a cyst nematode. *Genome Biol.* **15**, R43. (doi:10.1186/gb-2014-15-3-r43)

55. Boucher AC, Mimee B, Montarry J, Bardou-Valette S, Bélair G, Moffett P, Grenier E. 2013 Genetic diversity of the golden potato cyst nematode *Globodera rostochiensis* and determination of the origin of populations in Quebec, Canada. *Mol. Phylogenet. Evol.* **69**, 75–82. (doi:10.1016/j.ympev.2013.05.020)
56. Nei M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.
57. Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996–2004 GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).
58. Langella O. 2000 POPULATIONS 1·2: population genetic software, individuals or population distance, phylogenetic trees. See http://bioinformatics.org/~tryphon/populations.
59. Overall ADJ, Nichols RA. 2001 A method for distinguishing consanguinity and population substructure using multilocus genotype data. *Mol. Biol. Evol.* **18**, 2048–2056. (doi:10.1093/oxfordjournals.molbev.a003746)
60. R Core Team. 2014 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org.
61. Wang JL, Whitlock MC. 2003 Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**, 429–446.
62. Hedgecock D. 1994 Does variance in reproductive success limit effective population size of marine organisms? In *Genetics and evolution of aquatic organisms* (ed. A Beaumont), pp. 122–134. London, UK: Chapman and Hall.
63. Hedrick P. 2005 Large variance in reproductive success and the *Ne/N* ratio. *Evolution* **59**, 1596–1599. (doi:10.1111/j.0014-3820.2005.tb01809.x)
64. Tellier A, Lemaire C. 2014 Coalescence 2.0: a multiple branching of recent theoretical developments and their applications. *Mol. Ecol.* **23**, 2637–2652. (doi:10.1111/mec.12755)
65. Wang J, Santiago E, Caballero A. 2016 Prediction and estimation of effective population size. *Heredity* **117**, 193–206. (doi:10.1038/hdy.2016.43)
66. Williamson EG, Slatkin M. 1999 Using maximum-likelihood to estimate population size from temporal changes in allele frequencies. *Genetics* **152**, 755–761.
67. Birkner M, Blath J, Steinrucken M. 2011 Importance sampling for Lambda-coalescents in the infinitely many sites model. *Theor. Popul. Biol.* **79**, 155–173. (doi:10.1016/j.tpb.2011.01.005)
68. Montano V. 2016 Coalescent inferences in conservation genetics: should the exception become the rule? *Biol. Lett.* **12**, 20160211. (doi:10.1098/rsbl.2016.0211)
69. Caballero A, Hill WG. 1992 Effective size of nonrandom mating populations. *Genetics* **130**, 909–916.
70. Ghai GL. 1969 Structure of populations under mixed random and sib mating. *Theor. Appl. Genet.* **39**, 179–182. (doi:10.1007/BF00272526)
71. Waples RS. 2005 Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? *Mol. Ecol.* **14**, 3335–3352. (doi:10.1111/j.1365-294X.2005.02673.x)
72. Frankham R. 1995 Effective population size/adult population size ratios in wildlife: a review. *Genet. Res.* **66**, 95–107. (doi:10.1017/S0016672300034455)
73. Bartley D, Bagley M, Gall G, Bentley B. 1992 Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conserv. Biol.* **6**, 365–375. (doi:10.1046/j.1523-1739.1992.06030365.x)
74. Hoarau G, Boon E, Jongma DN, Ferber S, Palsson J, der Veer HWV, Rijnsdorp AD, Stam WT, Olsen JL. 2005 Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proc. R. Soc. B* **272**, 497–503. (doi:10.1098/rspb.2004.2963)
75. Barrière A, Félix MA. 2005 High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr. Biol.* **15**, 1176–1184. (doi:10.1016/j.cub.2005.06.022)
76. Sivasundar A, Hey J. 2005 Sampling from natural populations with RNAi reveals high outcrossing and population structure in *Caenorhabditis elegans*. *Curr. Biol.* **15**, 1598–1602. (doi:10.1016/j.cub.2005.08.034)
77. Cutter AD. 2006 Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer *Caenorhabditis elegans*. *Genetics* **172**, 171–184. (doi:10.1534/genetics.105.048207)
78. Archie EA, Ezenwa VO. 2011 Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. *Int. J. Parasitol.* **41**, 89–98. (doi:10.1016/j.ijpara.2010.07.014)
79. Fournet S, Kerlan MC, Renault L, Dantec JP, Rouaux C, Montarry J. 2013 Selection of nematodes by resistant plants has implications for local adaptation and cross-virulence: local adaptation and cross-virulence in *Globodera pallida*. *Plant Pathol.* **62**, 184–193. (doi:10.1111/j.1365-3059.2012.02617.x)
80. Barrett LG, Thrall PH, Burdon JJ, Linde CC. 2008 Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends Ecol. Evol.* **23**, 678–685. (doi:10.1016/j.tree.2008.06.017)
81. Nunney L, Luck RF. 1988 Factors influencing the optimum sex ratio in a structured population. *Theor. Popul. Biol.* **33**, 1–30. (doi:10.1016/0040-5809(88)90002-0)
82. Green CD, Greet DN, Jones FGW. 1970 The influence of multiple mating on the reproduction and genetics of *Heterodera rostochiensis* and *H. schachtii*. *Nematologica* **16**, 309–326. (doi:10.1163/187529270X00333)
83. Triantaphyllou AC, Esbenshade PR. 1990 Demonstration of multiple mating in *Heterodera glycines* with biochemical markers. *J. Nematol.* **22**, 452–456.
84. Wang J. 1996 Inbreeding and variance effective sizes for nonrandom mating populations. *Evolution* **50**, 1786–1794. (doi:10.1111/j.1558-5646.1996.tb03565.x)
85. Kimura M. 1962 On the probability of fixation of mutant genes in a population. *Genetics* **47**, 713–719.
86. Der R, Epstein C, Plotkin JB. 2012 Dynamics of neutral and selected alleles when the offspring distribution is skewed. *Genetics* **191**, 1331–1344. (doi:10.1534/genetics.112.140038)
87. Eldon B, Wakeley J. 2006 Coalescent processes when the distribution of offspring number among individuals is highly skewed. *Genetics* **172**, 2621–2633. (doi:10.1534/genetics.105.052175)
88. Turner SJ, Fleming CC. 2002 Multiple selection of potato cyst nematode *Globodera pallida* virulence on a range of potato species. I. Serial selection on *Solanum*-hybrids. *Eur. J. Plant Pathol.* **108**, 461–467. (doi:10.1023/A:1016018002152)
89. Phillips MS, Blok VC. 2008 Selection for reproductive ability in *Globodera pallida* populations in relation to quantitative resistance from *Solanum vernei* and *S. tuberosum* ssp. *andigena* CPC2802. *Plant Pathol.* **57**, 573–580. (doi:10.1111/j.1365-3059.2007.01771.x)
90. Montarry J, Bardou-Valette S, Mabon R, Jan P-L, Fournet S, Grenier E, Petit EJ. 2019 Data from: Exploring the causes of small effective population sizes in cyst nematodes using artificial *Globodera pallida* populations. Dryad Digital repository. (http://dx.doi.org/10.5061/dryad.7t3j55p)