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Adaptive response to olive cultivation ~~of~~ in a generalist parasitic nematode (*Meloidogyne javanica*)

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Running head: Parasite adaptive response to olive cultivation

ABSTRACT: Cultivated plants usually differ from their wild progenitors in several morphological and/or physiological traits. Their microbe communities might also differ ~~in particular due to~~because of adaptation to new conditions related to cultivation. To test this hypothesis, we ~~here~~ investigated morphological traits in a parthenogenetic root-knot nematode (*Meloidogyne javanica*) from natural and agricultural environments. Seventeen populations of *M. javanica* were sampled on cultivated ~~or~~ and wild olives in Morocco, and then maintained in control conditions for a "common garden" experiment. We estimated the genetic variation on three traits (stylet size, neck width, and body width) by a quantitative genetic design - ten families per population and nine individuals per family were measured - while molecular variation was investigated with a mitochondrial marker to identify the genetic lineages of nematode isolates sampled from wild and cultivated olives. Significant morphological differences were detected between individuals from wild *vs*. cultivated host for the three traits, while no phylogenetic clustering was observed among isolates collected on those two hosts. Our results thus ~~sustain~~ suggest an adaptive response of the ~~studied~~ asexual parasite, ~~likely for dealing with~~possibly related to the deep modification of soil nematode ~~community~~ communities between natural olive stands and orchards.

ADDITIONAL KEYWORDS: clonal evolution – cultivation – genetic – morphology – olive tree – *Olea europaea* – root-knot nematodes – *Meloidogyne javanica.*

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INTRODUCTION

The evolution of traits depends upon genetic variation on which natural selection can act to produce adaptations in response to the environment (Fabian & Flatt, 2012). Environmental variation modulates organismal development and substantially contributes to phenotypic variation within and among populations. Understanding how environmental and genotypic variations interact to generate phenotypic variation is thus a central challenge in biology, especially for deciphering how organisms adapt to anthropogenic disturbance (e.g. Braendle & Teotonio, 2015; Cook, 2018; Grishkan *et al.*, 2018; Ringot *et al.*, 2018). Domesticated organisms are appropriate models for the study of such evolutionary processes due to their recent evolution under selection (< 12,000 years ago for most crops), and good archaeological or historical records on their human-mediated diffusion and subsequent diversification (Meyer & Purugganan, 2013). Besides, novel environmental conditions (e.g. related to animal breeding or plant cultivation) may also have ecological and evolutionary consequences on the interacting biota. For instance, parasites and endophytes associated to plants or animals should have also adapted to their host after their domestication (e.g. Stukenbrock *et al.*, 2007; Gladieux *et al.*, 2010).

The olive tree (*Olea europaea* L.) is the iconic Mediterranean crop that accompanied the emergence of first civilizations in the Middle East (Kaniewski *et al.*, 2012). Archaeological evidences supports an early olive exploitation during the Neolithic, but its domestication (characterized by the establishment of orchards, and the vegetative multiplication of individuals with interesting agronomical traits, such as bigger fruits and adaptation to anthropogenic habitats) is considered to start in the Levant at the Chalcolithic period, at least 6000 years ago (for a review see Kaniewski *et al.*, 2012). The cultivated olive origins are complex and multiple, with the human-mediated diffusion of cultivars, first from the northern Levant, followed by recurrent admixtures with local wild olives (commonly referred to oleasters) or (pre-

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)domesticated forms in various parts of the Mediterranean Basin (Díez *et al.*, 2015; Besnard & Rubio de Casas, 2016). The structure of microbiome communities associated to the olive still needs to be better investigated to assess the impact of olive domestication and cultivation practices on their diversity (Besnard *et al.*, 2018). Recent studies on nematodes (Palomares-Rius *et al.,* 2012, 2015; Ali *et al.*, 2017; Archidona-Yuste *et al.,* 2018, 2020a) and fungi (Montes-Borrego *et al.*, 2014; Abdelfattah *et al.,* 2015) associated to the cultivated and/or wild olives indicate that such communities are influenced by environmental factors (e.g. soil parameters, cultivation methods) but also the genotype of the host. In particular, Ali *et al.* (2017) reported higher species richness for plant parasitic nematodes (PPNs) in wild olive stands, while their abundance was higher in olive orchards.

The mitotic parthenogenetic *Meloidogyne javanica* (Treub) Chitwood is one of the nematode species parasiting the olive (Ali *et al.*, 2014). Like all PPNs impacting the roots, *Meloidogyne* species prevent proper water and nutriments absorption. By consequence, the impact on growth and yield of olive can be important (Lamberti & Lownsbery, 1968; Lamberti & Baines, 1969; Sasanelli *et al.*, 1997, 2002), notably in nurseries (Nico *et al.*, 2002). While few studies have been carried out on the intra-population genetic variability or the genetic dispersal between populations in plant parasitic nematodes (Plantard & Porte, 2004), root-knot nematodes (or RKNs; *Meloidogyne* spp.) have been relatively well studied. A particular attention was given to RKNs because they are widely distributed and are endoparasites of nearly every species of higher plants (Moens *et al.*, 2009) causing significant yield crop losses worldwide. A huge morphological and genetic variability have been reported between *Meloidogyne* species and between populations from the same species (Netscher, 1978; Hesar *et al.*, 2011; da Silva *et al.*, 2014; Medina *et al.*, 2017; Tatu-Nyaku *et al.*, 2018). This diversity has been described based on various biological traits such as the morphology but it can also be studied through the ability of parasitism or the adaptation to environmental conditions (Jepson,

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1987; Eisenback & Triantaphyllou, 1991). In addition, the selection of virulent strains of RKNs on resistant plants may result in a co-evolution between the parasitic nematode and its host (Castagnone-Sereno, 2006).

The aim of this study was to evaluate the effect of crop cultivation on trait variation in one RKN species parasiting olive roots. We conducted a common garden experiment in order to measure the differentiation of three quantitative traits between 17 Moroccan populations of *M. javanica* collected in both cultivated and natural *O. europaea* stands. We also characterized genetic lineages of those RKNs using a mitochondrial marker, to control for their relatedness and assess lineage diversity within and among populations.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL AND FIELD SAMPLING

In this study, we worked on the 17 Moroccan populations of the nematode *Meloidogyne javanica* that were sampled in olive stands by Ali *et al.* (2016, 2017). Even if this species is parasiting the roots, we collected soil in the upper rhizosphere (the 20–30 cm deep) with a small spade (Ali *et al.*, 2016, 2017), because *M. javanica* spend almost all of their life cycle in the soil (Cadet & Thioulouse, 1998). This nematode survey was carried out from March to April 2012. Population locations (with GPS coordinates) are given in Fig. S1. We reused the same population labels as described in Ali *et al.* (2017). Those populations were either collected on cultivated olives or oleasters, to which we will then refer as "Cultivated" and "Wild" habitat, respectively [see Ali *et al.* (2017) for more details on sampling locations]. Cultivated olives from the agricultural sites correspond to the 'Picholine Marocaine' cultivar (Ali *et al.*, 2017), that is largely spread in Morocco (Khadari *et al.*, 2008), but we did not have any information about rootstock genotypes. All cultivated olives were conducted in traditional (7 locations) and

high density orchards (5 locations; Ali *et al.*, 2017). The soil sample was brought back in the quarantine laboratory at the Center for Biology and Management of populations (UMR CBGP, France). The species identification was first made morphologically (for the genera) and then was confirmed by esterase phenotype analysis (Ali *et al.*, 2016). *Meloidogyne javanica* populations were then reared and maintained on tomato. For nematode inoculation, tomato plants (*Lycopersicon esculentum* Mill. *cv.* Roma) were prepared in 50-ml plastic corning tubes (35-mm diameter) containing a mixture of compost, sand and sodium polyacrylamide crystals.

Characterization of maternal lineages with a mitochondrial DNA sequence

We first controlled for the relatedness and lineage diversity within and among *M. javanica* populations. For this purpose, we collected individuals of *M. javanica* reared on tomatoes (Fig. 1) just after field collection as explained above. An individual second stage juvenile (J2) was isolated from fifteen egg masses per population and then conserved in 6 µL of distilled water at -20°C. DNA was then extracted from each J2 using the DNeasy Blood & Tissue kit (Qiagen Inc.). Because a small amount of living material was used, DNA extracts were poorly concentrated (< 1 ng/µL).

Considering the lack of suitable genetic markers for *Meloidogyne*, knowledge of their population genetic structure and colonization process remains poor. Given the asexual reproductive mode of *M. javanica* and its relatives (i.e. obligate parthenogenesis; Castagnone-Sereno, 2006), we expect that clonal reproduction led to no sexual recombination and an overall low genomic variation between isolates, as recently reported in a close relative (*M. incognita*; Koutsovoulos *et al.*, 2020). Mitogenome (mtDNA) polymorphisms allowing distinguishing species and lineages among parthenogenetic RKNs of the *M. incognita* group (MIG group; Janssen *et al.*, 2016), we developed an mtDNA marker based on a highly variable minisatellite. A non-coding region containing 63-bp tandem repeats, named 63R in *M. javanica* (Okimoto *et al.*, 1991; Besnard *et al.*, 2014), was thus sequenced. The use of an mtDNA locus also had the

advantage of an easier PCR amplification (compared to nuclear single-copy regions) due to multiple copies in the cell. Such a locus is thus a marker of choice when dealing with poorly concentrated DNA extracts. Although the mitogenome is supposedly non recombinant, heteroplasmy (i.e. mix of mtDNA variants within individuals) has however been reported in *Meloidogyne* (especially in the 63R minisatellite, but not only; Okimoto *et al.*, 1991; Besnard *et al.*, 2019). Beforehand, such intra-individual variation needs to be carefully considered because it may limit the usefulness of mtDNA polymorphisms for population genetics (Lunt *et al.*, 1998).

The 63R fragment was amplified by PCR with the following primers: 63R-For1 (TTGAAATTGCTTTATTGTTACTAAGAAG) and 63R-Rev (ACCTTAGAAATATGAAWCCTAWAGA). PCR reaction assays were carried out in a 20-µL final volume containing 2 µL PCR buffer 10x (Qiagen), 1 µL of each primer (initial concentration at 10 µM), 12 µL of UV treated distilled water and 4 µL of genomic DNA. The thermocycler was programmed for 15 min at 95°C followed by 40 cycles of 30 s at 94°C, 1 min, 30 s at 55°C and 1 min 30 at 72°C and then 10 min at 72°C. PCR products were purified and sequenced in both directions using the same amplification primers referred above by Eurofins MWG (Germany). When necessary (i.e. fragment size > 800 bp), a third sequencing reaction was performed with an internal primer (63R-For2: GAATTCCATAAATCAGAAAATTGAGG). In total, we successfully analyzed 119 individuals from both natural (37 individuals) and agricultural locations (82 individuals; Table S1).

Sequences were manually edited with the BioEdit software (Hall, 1999). We noticed cases of heteroplasmy when a mix of distinct length or single nucleotide variants was observed in the chromatogram. In such cases (ca. 10% of isolates), the heteroplasmic pattern was carefully annotated and the most abundant sequence was considered (see results). Sequences were finally aligned in MEGA v.6 (Tamura *et al.*, 2013). Mitotype networks were reconstructed

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with the reduced median method implemented in NETWORK v.10 (Bandelt *et al.*, 1999) considering only single nucleotide polymorphisms (that allowed identifying 'haplogroups'). Mitotypes of two closely related species [*M. arenaria* (*Ma*; LS974734) and *M. incognita* (*Mi*; KJ476151)] were used as outgroups. Length polymorphisms due to variable number of repeats in the minisatellite (that allowed identifying 'haplotypes') were secondarily considered, but as this repeated motif shows a relatively high level of heteroplasmy, it was interpreted with caution (see results).

Population structure was analyzed using hierarchical *F*-statistics, estimated from variance components of gene frequencies (Weir & Cockerham, 1984). F_{ST} is a metric of population differentiation at neutral genetic markers (Weir & Cockerham, 1984) and here was calculated between habitats (populations are nested within habitat i.e. cultivated *vs.* wild, and individuals are nested within population) with the package HIERFSTAT (Goudet, 2005) for the statistical software R (R Development Core Team, 2019). For each population, we also estimated an index of mtDNA diversity as $D = 1 - \Sigma p_i^2$, where p_i is the frequency of haplogroup or haplotype i in the population. This parameter was thus calculated for both mtDNA haplogroups ($D_{haplogroups}$) and haplotypes ($D_{haplotypes}$).

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EXPERIMENTAL DESIGN CONSTRUCTION

For each *M. javanica* population, egg masses were randomly selected from ten mothers (after molecular characterization, which was done on different individuals; Fig. 1) and put individually to hatch in distilled water in Petri dishes (5-cm diameter). This species reproduces by obligatory mitotic parthenogenesis, i.e. females reproduce without the involvement of males or sperm (Van der Beek *et al.*, 1998). This means that all individuals coming from the same mother are expected genetically identical between them and also to their mother. Each egg mass is considered as progeny (i.e. family). Ten egg masses (i.e. ten families) were thus formed per population. From each egg mass, 50-60 infective juveniles (J2) were withdrawn by a pipette

and used to inoculate a one-month-old tomato plant. The inoculum suspension was injected in several holes onto the soil surface around the stem base. The plants were maintained in a climate chamber with 12 hours light at 23°C and 12 hours dark at 20°C.

Four to six weeks after inoculation, egg masses of each family were isolated from each tomato plant (Fig. 1). Three egg masses per plant were collected representing three replicates per family. Egg masses were individually put to hatch in Petri dishes (5-cm diameter), and three individuals per egg mass were then randomly chosen for further phenotypic measurements (see below). The dishes were controlled every day in order to take individuals for morphological measurements at the same age. This was necessary to avoid a spurious relationship between the morphological variability among individuals with their stage of development. Fixing was thus done when individuals were ten-days old (after hatching). Freshly hatched second-stage juveniles (J2s) were randomly picked from each dish and placed in a drop of water. J2s were temporarily conserved in the fridge and then fixed and killed in the glycerol previously heated at 40°C (De Grisse, 1969).

Traits measurements and analyses

For the morphometric measures, three J2s per egg mass were collected and deposited in a drop of 15 µL of fixing solution on a slide with paraffin wax. The slide was then covered with a glass cover slip and then placed for a few seconds on a hot plate to ensure that the paraffin melts and then left to cool to keep the J2s trapped in the fixing solution. More than 1530 individuals were measured as described in Fig. 1. Several photos were performed on each J2. All observations were carried out under an optical microscope (Leica DMRD) attached to a camera (Leica DMF 20). Measurements were then done on photos using the LAS (Leica® Application Suite) applied software v.2.5.0 R1. On each J2, the following morphological traits were measured (Fig. 2): (*i*) maximum body width; (*ii*) width of the body at the median bulb (hereafter neck width); and (*iii*) stylet length. Measures of these traits are frequently used in the morphological

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characterization of RKNs, and an intraspecific quantitative variation has been reported (Jepson, 1987; Siddiqi, 2000; Hesar *et al.*, 2011). The observations were made under magnification of 10× or 20× for the entire size of the individual, and under 100× for the two others characters. A Spearman test (Hall, 2015) was used to assess the correlation between the three traits.

We considered traditional and high-density orchards together as "cultivated" locations. We tested the values of traits between habitats ('Cultivated' or 'Wild') using a hierarchical analysis of variance (ANOVA) using the statistical software R (R Development Core Team, 2019). Populations were thus nested into habitat, families into population, and individuals into families. By this way, we estimated the variance components between habitats (σ^2_{Hab}), between populations within habitats (σ^2_{Pop}), between families within populations (σ^2_{Fam}) and between individuals within families (σ^2_{Ind}) with the method of restricted maximum likelihood (REML; Lynch & Walsh 1998). Individuals are thus nested within a family, which is nested within a population, which is nested within a habitat. REML estimates were obtained using the package NLME for R (Pinheiro *et al.*, 2019). These components of variance allowed us to estimate broad-sense heritability (H^2) for each phenotypic trait. H^2 represents the amount of the genetic part in the phenotypic variation of a given trait. Heritability scores range from 0 to 1; when $H^2 = 1$, then all variation in a population is due to differences or variation between genotypes (i.e. there is no environmentally caused variation); at the opposite, when $H^2 = 0$, all variation in the population comes from the environment experienced by individuals. Although there can be a range depending on the methodology used, heritability values less than 0.20 are usually considered low, between 0.21 and 0.40 are moderate, and above 0.40 are high (Lynch & Walsh, 1998). Broad-sense heritabilities were calculated as $H^2 = V_G / V_P$, with V_G and V_P the population genetic and phenotypic variances, respectively (Falconer, 1989). Since individuals reproduced by parthenogenesis, offspring are considered genetically identical to their mother. Thus, genetic variation (V_G) was estimated as the between-family variance (σ^2_{Fam}), and V_E was estimated by

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3 the between-individuals variance (σ^2_{Ind}). V_P is the result of the genetic plus the environmental variances. Barton & Turelli (1989) argued that common garden experiments like here can overestimate heritabilities because the environmental variance is greatly reduced. In order to investigate genetic variation without any environmental variance, Houle (1992) suggested using the coefficient of genetic variation (CV_G): $CV_G = (\sigma^2_{Fam})^{0.5} / m$, where m is the population phenotypic mean.

Q_{ST} is the analog of F_{ST} but for quantitative trait. It measures differentiation at a quantitative trait (Spitze, 1993; Whitlock, 2008). We thus estimated quantitative trait differentiation among habitats for each trait as the quantitative variance between habitats over the total [i.e. $\sigma^2_{Hab} / (\sigma^2_{Hab} + \sigma^2_{Pop} + \sigma^2_{Fam})$].

A Mantel test was also used to evaluate if the morphological variability between populations is correlated with the geographical distance between populations. We computed this test using the library vegan (Dixon, 2003) in R.

At last, we tested a possible correlation between molecular diversity (D) and the quantitative diversity for each population (considering either σ^2_{Fam}, H^2 or CV_G). In particular, we tested that a population with high molecular diversity could also show a high quantitative diversity. Correlations between diversity indexes ($D_{haplogroups}$ or $D_{haplotypes}$) and quantitative diversity parameters were estimated using the Pearson method (Hall, 2015) in R. However, as we tested multiple correlations for three traits, the problem of multiplicity occurs. Indeed, the more hypotheses we check, the higher the probability of finding a significant p-value by chance arises. We thus « corrected » those p-values by the « Bonferroni correction » in which the p-values are multiplied by the number of comparisons.

RESULTS

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In this study, we collected 17 *M. javanica* populations in Morocco: 12 from cultivated conditions and five from natural conditions (Fig. S1). Populations belonging to the same habitat ('Cultivated' *vs.* 'Wild') are not significantly closer to one another than populations belonging to different habitats (Mantel test : R = 0.10 and $p = 0.16$). In other words, there is no correlation between the habitat type and the geographical distance.

MOLECULAR VARIATION

An mtDNA fragment (63R region) of 900 to 1400 bp was generated on 119 individuals. Sequencing revealed variation in the number of repeats in the minisatellite 63R region plus five additional single nucleotide polymorphisms (SNPs) located in regions flanking the 63R. A careful examination of these SNPs showed one case of heteroplasmy on individual '253-M6' that combined two types of sequences [referred to *Mj* I-7 (the most abundant; above 80%) and *Mj* IV-7; Table S1]. Other accessions did not show such patterns of intra-individual polymorphisms on SNPs, indicating that these polymorphisms may be potentially informative to distinguish divergent evolutionary lineages of *M. javanica*. These five SNPs allowed the distinction of four mitochondrial haplogroups (Fig. 3). Haplogroup *Mj* III was only found at location P-285, and is relatively divergent from the others (distinguished by three or four SNPs). Individuals collected on wild and cultivated olives shared the two most frequent related haplogroups *Mj* I and *Mj* II (> 40%; Table 1), while two minor haplogroups (< 10%) were only found in two cultivated locations (P-253 and P-285; Fig. 3). No clear distinction between isolates collected on wild and cultivated olives was thus observed based on SNPs.

When considering the number of repeats within the 63R minisatellite, a higher variation was observed (Figs S2, S3). The number of repeats varied from 7 to 14. By combining SNPs and length variation, 19 haplotypes were distinguished (Tables S1 and S2; GenBank nos: LS974735 to LS974753) but a relatively high level of heteroplasmy was also observed (13/119 isolates; Table S1). In addition, the haplotype network was mostly unresolved (Fig. S2)

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indicating a high level of homoplasy in the data. For these reasons, their interpretation has to be done with caution. Overall, the relatively high intra-population diversity (Fig. S3) coupled to the fact that many close locations did not share any haplotypes nevertheless suggest a complex pattern of isolation and migration between locations, but this needs to be investigated with more reliable DNA markers on a larger population sampling. Yet, isolates from wild and cultivated olives again shared numerous haplotypes indicating they do not represent old diverging evolutionary lineages.

We found no genetic structure between habitats (F_{ST} = -0.044 and -0.017, for haplogroups and haplotypes, respectively), while a relatively high structure was detected between populations within habitat (F_{ST} = 0.516 and 0.464, using haplogroups and haplotypes, respectively).

Phenotypic variation

The mean trait values per population are reported in Table 2, and are similar to those previously reported on *M. javanica* (e.g. Hesar *et al.*, 2011). One weak correlation was found between neck and body widths (R = 0.10, $p < 0.05$). Individuals belonging to populations parasiting cultivated olive trees tend to be smaller. They exhibit a smaller body width, a smaller neck and a smaller stylet (Table 2). They all show a significant difference between the 'Cultivated' and 'Wild' conditions (Table 2). Between habitats, mean broad-sense heritabilities are varying across traits, ranging from 0.13 to 0.30 (Table 3). Mean CV_G values between habitats are low and range from 0.01 to 0.02. Values per population are given in Table S3.

Q_{ST} values were estimated between habitats for the three traits (Table 4). They range from 0.04 to 0.48, suggesting a structure in phenotypic traits between habitats, i.e. wild *vs.* cultivated hosts. Q_{ST} values were relatively high for the neck and body widths (0.31 and 0.48, respectively), in contrast to the size of the stylet, for which the measured Q_{ST} was very low

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(0.04). No significant correlation between molecular diversity and quantitative values per population for all traits was detected (Table S3; Figs S4, S5).

Because our sample design was unbalanced (i.e. 12 and five populations respectively sampled on cultivated and wild olives), some of our comparisons could be biased. We thus chose to compare the five populations sampled on high-density cultivated olives against the five populations sampled on oleasters. The results are shown in supplementary material (Tables S4, S5, S6) and exhibit very similar results suggesting that our results based on the whole sample are reliable for molecular and quantitative data.

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DISCUSSION

In this study, molecular and morphological variation was observed among isolates of a plant-parasitic nematode of the olive tree. We first checked that there is no genetic isolation between populations sampled in "Cultivated" and "Wild" habitats. After growing in controlled conditions, we found that populations collected from olive orchards are smaller (for stylet size, body and neck widths) than in oleaster stands. We then quantified the genetic basis of such phenotypic differences. A selective process was suggested by our observations despite the clonal reproductive mode of *M. javanica*. Our results ~~sustain~~ suggest an adaptive response of ~~this~~ the root parasite for dealing with environmental changes between natural olive stands and orchards.

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ON THE *M. JAVANICA* ASEXUAL EVOLUTION AND POPULATION DIFFERENTIATION

While low intraspecific genetic variation has been previously reported in *Meloidogyne* species (Castagnone-Sereno, 2002; Castagone-Sereno *et al*., 2019; Besnard *et al.*, 2019), a full nuclear genome analysis recently brought strong insights for clonal evolution in the parthenogenetic *M. incognita* (Koutsovoulos *et al*., 2020). Yet, this species kept a high adaptive potential despite its clonal mode of reproduction, and a similar pattern may be expected in *M. javanica* that also belongs to the MIG group (Tigano *et al.*, 2005). Such asexual evolution is not common in animals and may have deep consequences on adaptive responses.

In our study, we were able to distinguish four main mtDNA haplogroups in *M. javanica* (and a total of 19 haplotypes when considering minisatellite variation; Table S2). We thus confirm the utility of the mitogenome for distinguishing lineages among the MIG group, even at the species level (Janssen *et al.*, 2016). Heteroplasmy was also detected (notably based on SNPs on one isolate). Such a mix of mitochondrial variants within some individuals may either result from the accumulation of mutations and maintenance of DNA polymorphisms over time, or some exchange of genetic material between individuals (possibly via horizontal gene transfers, demonstrated between distantly related species, but that could also happen between congeners; Danchin, 2011; Dunning & Christin, 2020). Whatever its origin, heteroplasmy has to be considered with caution, and we thus put more emphasize on SNPs, for which heteroplasmy was rare (< 1%). SNPs first allowed us to demonstrate that populations sampled on cultivated or wild olives are not differentiated. This means that *M. javanica* populations may exchange individuals between habitats. In contrast, we however showed a relatively high differentiation between populations within habitats, suggesting patterns of rapid isolation at the local scale, probably due to very stochastic events of extinction and dispersal via infected plant, soil or water [e.g. close populations 261 and 262 from the "Cultivated" habitat do not share any mitochondrial haplogroups (Fig. 3), while many haplotypes are specific to some populations

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(Fig. S3)]. Despite the supposed clonal evolution in *M. javanica*, an unexpected high mtDNA variation was thus observed within and among populations in both natural and cultivated habitats (Fig. 3). Distinct nematode lineages therefore co-occur within both habitats, which may maintain an evolutionary potential for adapting to variable conditions.

Diverging selection between habitats

In parallel, significant phenotypic difference was observed between habitats strongly suggesting local adaptation of *M. javanica* to "Cultivated" or "Wild" conditions. Here, low to moderate heritability and CV_G values reveal a genetic basis to this variation. However, while such morphological difference can be explained by selection, drift could also be responsible for this pattern. In this context, comparisons of population differentiation at quantitative traits (Q_{ST}) with population differentiation at genetic markers (F_{ST}) can provide a powerful tool (Merilä & Crnokrak, 2001; McKay & Latta, 2002; Leinonen *et al.*, 2008; Whitlock, 2008). Selection is expected to affect regions at or close to the quantitative trait loci underlying the phenotypic trait, while neutral processes should have equal chance to affect any genomic region. To be short, a phenotypic differentiation exceeding neutral differentiation (i.e. $Q_{ST} > F_{ST}$) is a sign of directional selection (Merilä & Crnokrak, 2001). Here, we found no phylogenetic clustering among isolates collected in cultivated and natural conditions with a null F_{ST} value between habitats, while Q_{ST} values for the weakly correlated neck and body widths were relatively high (0.31 and 0.48, respectively). This pattern ($Q_{ST} > F_{ST}$) thus supports a selective effect on morphological divergence not due to drift alone. In contrast, we surprisingly did not detect a local adaptation signal between habitats for the size of the stylet, which is yet an important appendix for nematode nutrition. Environmental and genetic variances were however higher within the cultivated habitat than in the natural habitat. This absence of signal is possibly a consequence of a limited sampling in the wild, but another explanation could be the relaxation of selection on the stylet length due to a reduced inter-specific competition in the cultivated

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habitat where the nematode communities are less diverse compared to the natural habitat (Ali *et al.*, 2017). We should thus investigate how this trait behaves during intra- and inter-specific competition. In addition, we cannot exclude that the stylet size Q_{ST} is also influenced by non-additive components due to dominance and epistasis (Goudet & Büchi, 2006).

With its cultivation by humans, the environment of the olive has been considerably modified (via various cultural practices, including weed removal, nutrition and the use of pesticides), indirectly affecting phenotypes and community composition of microbes associated to the species (e.g. Ali *et al.*, 2017). There is accumulating evidence on the ability of soil microbe communities to deal with such anthropogenic disturbances (e.g. Grishkan *et al.*, 2018; Holterman *et al.*, 2019), but this needs to be better documented to evaluate their role in the domestication process (Milla *et al.*, 2015). A different composition of nematode communities was recently reported between cultivated and natural habitats of the olive tree in Morocco and Spain (Ali *et al.*, 2017; Archidona-Yuste *et al.*, 2020a). While cultivated conditions affect nematode richness and diversity, this can be due to many factors such as soil composition, cultural practices or the cultivated genotype. For instance, resistance to *M. javanica* has been reported in some olive genotypes (Palomares-Rius *et al.*, 2019; Archidona-Yuste *et al.*, 2020b), while crop intensification and grafting practices may have deep consequences on soil nematode communities (e.g. Warschefsky *et al.*, 2016; Ali *et al.*, 2017; Barazani *et al.*, 2017). In the 17 locations here studied, Ali *et al.* (2017) showed a slightly lower phytoparasitic nematode (PPN) richness in cultivated stands than in the wild habitat (mean: 6.0 *vs.* 6.4 genera/dm^3), while, in contrast, the total PPN abundance increased (mean: 3977 *vs.* 3448 individuals/dm^3)]. This trend is in accordance with the general pattern reported in the study on 94 sites. The size reduction of *M. javanica* in cultivated conditions could thus be related to higher PPN density in this habitat. To deepen this hypothesis, measuring traits on several cohabiting nematode species would surely give insights on the structure and adaptive response of these communities in

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natural and cultivated habitats (e.g. displacement of characters for the coexistence of different species). We also recommend increasing the sampling and focusing on different types of orchards by controlling for a maximum of variables such as tree density, irrigation, crop genotype diversity (for both rootstocks and scions) and soil characteristics for testing their impact.

What determines an adaptive response in an asexual nematode?

Finally, our study highlighted an adaptive response in an asexual parasite. This ~~could be~~ might be unexpected since clonal reproduction should lead to limited genomic recombination. Nevertheless, genomic plasticity of RKNs should produce some diversity that is essential for their evolutionary response (Castagnone-Sereno & Danchin, 2014). This may be promoted via lateral gene transfers and large genomic rearrangements (Danchin, 2011; Koutsovoulos *et al.*, 2020). An epigenetic response should be also investigated because this could be responsible for heritable variation in gene expression (e.g. Mirouze & Paszkowski, 2011). Such exhaustive phenotypic and genomic characterization of *M. javanica* populations parasiting olives may be very informative to test these hypotheses, but as shown in our study, its implementation at large scale still remains a technical challenge (i.e. both for settling controlled experiments and generating genomic data).

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SUPPORTING INFORMATION

Additional supporting Information may be found on the online version of this article at the publisher's website:

Table S1. List of the 119 isolates characterized with the 63R region.

Table S2. Mitotype occurrence in individuals isolated from cultivated and wild olives.

Table S3. Quantitative components of variance for three phenotypic traits.

Table S4. F_{ST} values among habitats and between populations of *Meloidogyne javanica* when considering only high-density cultivated and wild olive populations.

Table S5. Effect of habitat, population, and family on three life-history traits of *Meloidogyne javanica* sampled in high-density cultivated or wild olive stands.

Table S6. REML variance components for three quantitative traits measured on *Meloidogyne javanica* populations sampled in high-density cultivated and wild olive habitats.

Figure S1. Distribution of *Meloidogyne javanica* populations sampled in Morocco.

Figure S2. Mitochondrial haplotype reduced-median network.

Figure S3. Geographic distribution of mitochondrial haplotypes of *Meloidogyne javanica* in wild and cultivated olive habitats in Morocco.

Figure S4. Correlation plot between haplogroup diversity and quantitative phenotypic diversity calculated for the stylet size, neck width and body width.

Figure S5. Correlation plot between haplotype diversity and quantitative phenotypic diversity calculated for the stylet size, neck width and body width.

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Table 1. Mitochondrial haplogroup frequency in individuals isolated from cultivated and wild olives. Mitochondrial haplogroups were defined based on five SNPs (Fig. 3).

Mitotype	**Cultivated**	**Wild**
Mj I	45.1%	58.3%
Mj II	43.9%	41.7%
Mj III	8.6%	-
Mj IV	2.5%	-

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Table 2. Mean values of three life-history traits (measured in µm) studied on *Meloidogyne javanica* individuals from Cultivated (C) and Wild (W) olive stands (definition in text; locations given in Fig. S1). Standard deviation of each value is given in parentheses. The effects of habitat (Cultivated or Wild), population, and family (nested within population) were tested by hierarchical ANOVA. *p*-values are considered significant when inferior to 0.05, and coded as follow: *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$.

Trait	**C**	**W**	**Habitat**	**Population**	**Family**
Stylet size	12.85 (0.61)	13.05 (0.52)	***	***	*
Neck width	11.67 (0.38)	11.86 (0.33)	***	***	***
Body width	13.08 (0.71)	13.51 (0.61)	***	*	***

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Table 3. Quantitative components of variance for three phenotypic traits (stylet size, neck width and body width). These parameters (σ^2_{Fam}, σ^2_{Ind}, H^2, CV_G) were estimated per population and then used for calculating means by habitat (C = Cultivated; W = Wild) and on all samples (All).

Trait	Habitat	σ^2_{Fam}	σ^2_{Ind}	H^2	CV_G
Style size	C	0.0820	0.2031	0.2789	0.0199
	W	0.0680	0.0932	0.3280	0.0168
	All	0.0778	0.1708	0.2933	0.0190
Neck width	C	0.0140	0.1014	0.1373	0.0093
	W	0.0147	0.1034	0.1102	0.0095
	All	0.0142	0.1019	0.1305	0.0093
Body width	C	0.0867	0.4279	0.1846	0.0205
	W	0.0352	0.3179	0.0939	0.0131
	All	0.0716	0.3955	0.1579	0.0183

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Table 4. REML variance component for quantitative traits between habitats. For each trait, we report the variance between habitats (σ^2_{Hab}), between populations within habitat (σ^2_{Pop}), between families within populations (σ^2_{Fam}) and between individuals within families (σ^2_{Ind}). We also assessed the quantitative differentiation between habitats (Q_{ST}) of each trait (details in text).

Trait	σ^2_{Hab}	σ^2_{Pop}	σ^2_{Fam}	σ^2_{Ind}	Q_{ST}
Stylet size	0.008	0.110	0.080	0.171	0.040
Neck width	0.014	0.019	0.014	0.103	0.310
Body width	0.085	0.012	0.085	0.379	0.479

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Legends of Figures

Figure 1. Experimental design for molecular characterization and trait measurements of *M. javanica* populations. For each population, fifteen egg masses (one individual per egg mass) were randomly chosen for molecular measures, while ten egg masses were collected (families), and reared on tomatoes for the morphological measures. Then, for each family in every population, three individuals per egg-mass were randomly sampled and measured after photography (details on traits in the text). As we worked on 17 populations, ten families per population and nine individuals (on average) per family, 1530 nematodes were measured for three traits.

Figure 2. Photography of a nematode (under microscope; 100×) indicating the three traits measured and their morphological significance.

Figure 3. Mitochondrial DNA variation among *Meloidogyne javanica* isolates sampled on wild and cultivated olives in Morocco. Mitochondrial haplogroups were defined based on five single nucleotide polymorphisms (SNPs) in regions flanking the 63R minisatellite. **a)** Mitochondrial haplogroup reduced-median network (Bandelt *et al.*, 1999). Mitotypes of two closely related species [*M. arenaria* (*Ma*; LS974734) and *M. incognita* (*Mi*; KJ476151)] were used as outgroups (small black circles). Four haplogroups were distinguished among *M. javanica* isolates collected on cultivated olives, with the most two frequent ones (*Mj* I and *Mj* II) also observed on wild olive isolates (W). Size of pie charts is proportional to the relative frequency of each mitotype. **b)** Geographic distribution of haplogroups among the 17 provenances (between five and ten isolates were analyzed per location). Size of pie charts is proportional to the number of isolates analyzed per location. For mtDNA haplotypes defined on both SNPs and minisatellite length variation, see Figs S2 and S3.

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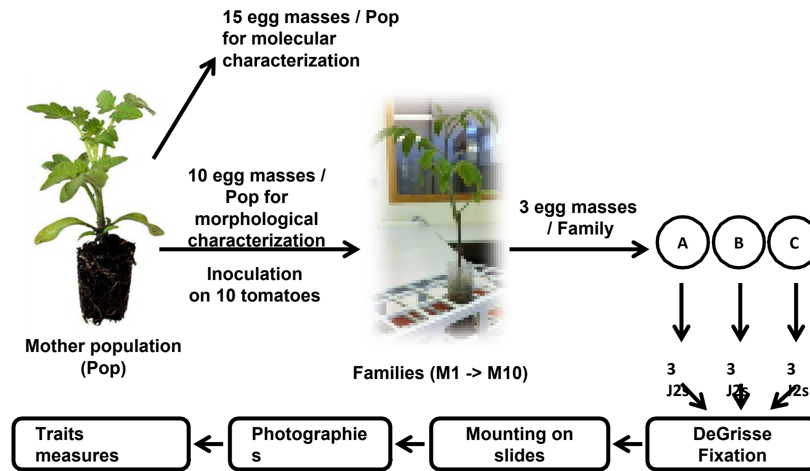

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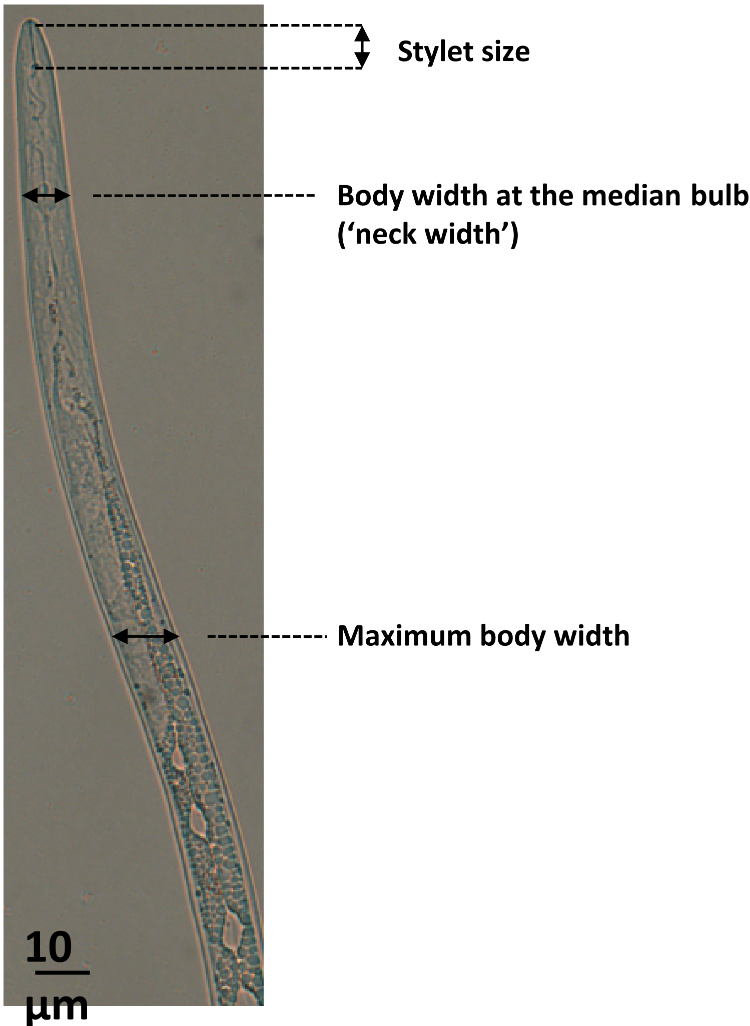

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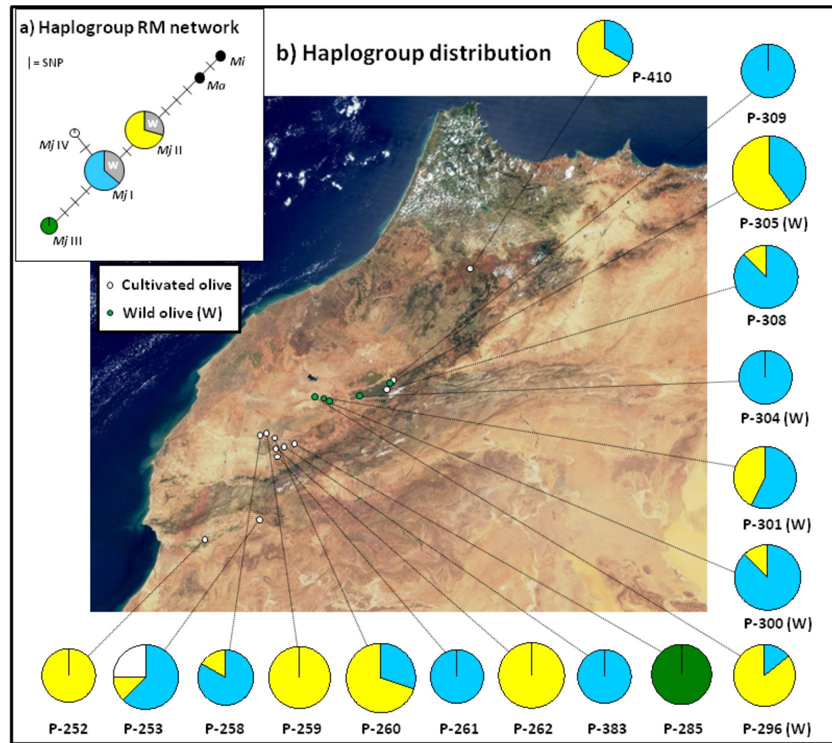

Figure 3. Mitochondrial DNA variation among Meloidogyne javanica isolates sampled on wild and cultivated olives in Morocco.

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SUPPORTING INFORMATION

Adaptive response to olive cultivation of a generalist parasitic nematode (*Meloidogyne javanica*)

ELODIE CHAPUIS, NADEEN ALI, CAMILLE NOUS & GUILLAUME BESNARD

Supporting Information contains:

Table S1. List of the 119 isolates characterized with the 63R region

Table S2. Mitotype occurrence in individuals isolated from cultivated and wild olives

Table S3. Quantitative components of variance for three phenotypic traits

Table S4. F_{ST} values among habitats and between populations of *Meloidogyne javanica* when considering only high-density cultivated and wild olive populations

Table S5. Effect of habitat, population, and family on three life-history traits of *Meloidogyne javanica* sampled in high-density cultivated or wild olive stands

Table S6. REML variance components for three quantitative traits measured on *Meloidogyne javanica* populations sampled in high-density cultivated and wild olive habitats

Figure S1. Distribution of *Meloidogyne javanica* populations sampled in Morocco

Figure S2. Mitochondrial haplotype reduced-median network

Figure S3. Geographic distribution of mitochondrial haplotypes of *Meloidogyne javanica* in wild and cultivated olive habitats in Morocco

Figure S4. Correlation plot between haplogroup diversity and quantitative phenotypic diversity calculated for the stylet size, neck width and body width

Figure S5. Correlation plot between haplotype diversity and quantitative phenotypic diversity calculated for the stylet size, neck width and body width

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Table S1. List of the 119 isolates characterized with the 63R region. SNPs allow us to distinguish four mitochondrial haplogroups, namely *Mj I*, *Mj II*, *Mj III* and *Mj IV*. Then, the number of repeats (from 7 to 14) in the 63R minisatellite is also given, allowing defining a mitochondrial haplotype. For 13 individuals with heteroplasmy (mix of at least two types of sequence), the main mitotype is defined as the most abundant sequence in the chromatogram. In these cases, minor sequences are also indicated in parenthesis.

Population ID	Habitat	Isolate	63R type
252	Cultivated	252M1	*Mj* II-11
252	Cultivated	252M2	*Mj* II-10
252	Cultivated	252M3	*Mj* II-10(11)
252	Cultivated	252M4	*Mj* II-10
252	Cultivated	252M5	*Mj* II-10
252	Cultivated	252M6	*Mj* II-10
253	Cultivated	253M1	*Mj* I-8
253	Cultivated	253M2	*Mj* IV-7
253	Cultivated	253M3	*Mj* I-8
253	Cultivated	253M5	*Mj* I-7(11,12,15)
253	Cultivated	253M6	*Mj* I(IV)-7
253	Cultivated	253M7	*Mj* IV-7
253	Cultivated	253M8	*Mj* II-9(10)
253	Cultivated	253M9	*Mj* I-14
258	Cultivated	258M1	*Mj* I-9
258	Cultivated	258M2	*Mj* I-9
258	Cultivated	258M3	*Mj* I-9
258	Cultivated	258M5	*Mj* I-9
258	Cultivated	258M7	*Mj* I-9
258	Cultivated	258M24	*Mj* II-9(11,13,16)
259	Cultivated	259M2	*Mj* II-13
259	Cultivated	259M3	*Mj* II-13
259	Cultivated	259M4	*Mj* II-12(13)
259	Cultivated	259M5	*Mj* II-13
259	Cultivated	259M6	*Mj* II-13
259	Cultivated	259M8	*Mj* II-13
259	Cultivated	259M9	*Mj* II-13
260	Cultivated	260M1	*Mj* II-8
260	Cultivated	260M2	*Mj* II-8(9)
260	Cultivated	260M5	*Mj* II-8
260	Cultivated	260M5	*Mj* II-8
260	Cultivated	260M7	*Mj* II-8
260	Cultivated	260M8	*Mj* II-8
260	Cultivated	260M17	*Mj* II-8
260	Cultivated	260M18	*Mj* I-7
260	Cultivated	260M19	*Mj* I-7
260	Cultivated	260M20	*Mj* I-7
261	Cultivated	261M3	*Mj* I-12
261	Cultivated	261M4	*Mj* I-12
261	Cultivated	261M5	*Mj* I-12
261	Cultivated	261M6	*Mj* I-12
261	Cultivated	261M7	*Mj* I-12

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Table S1, continued.

Population ID	Habitat	Isolate	63R type
262	Cultivated	262M1	*Mj* II-13
262	Cultivated	262M2	*Mj* II-9
262	Cultivated	262M3	*Mj* II-13
262	Cultivated	262M4	*Mj* II-13
262	Cultivated	262M5	*Mj* II-13
262	Cultivated	262M6	*Mj* II-13
262	Cultivated	262M7	*Mj* II-9
262	Cultivated	262M8	*Mj* II-9
262	Cultivated	262M9	*Mj* II-14
285	Cultivated	285M1	*Mj* III-8
285	Cultivated	285M2	*Mj* III-11(8)
285	Cultivated	285M3	*Mj* III-10
285	Cultivated	285M4	*Mj* III-11
285	Cultivated	285M5	*Mj* III-10
285	Cultivated	285M6	*Mj* III-8
285	Cultivated	285M7	*Mj* III-8
296	Wild	296M1	*Mj* II-8
296	Wild	296M2	*Mj* I-8
296	Wild	296M4	*Mj* II-8
296	Wild	296M5	*Mj* II-9
296	Wild	296M6	*Mj* II-9
296	Wild	296M7	*Mj* II-8
296	Wild	296M22	*Mj* II-13
300	Wild	300M1	*Mj* I-8
300	Wild	300M2	*Mj* I-8
300	Wild	300M5	*Mj* I-9
300	Wild	300M6	*Mj* I-8
300	Wild	300M7	*Mj* II-9
300	Wild	300M8	*Mj* I-8
300	Wild	300M9	*Mj* I-8
300	Wild	300M15	*Mj* I-8
301	Wild	301M1	*Mj* I-14(11)
301	Wild	301M2	*Mj* I-10
301	Wild	301M3	*Mj* I-12
301	Wild	301M4	*Mj* II-10
301	Wild	301M5	*Mj* II-10
301	Wild	301M6	*Mj* I-10
301	Wild	301M8	*Mj* II-10
304	Wild	304M1	*Mj* I-8
304	Wild	304M2	*Mj* I-8
304	Wild	304M3	*Mj* I-8
304	Wild	304M4	*Mj* I-8
304	Wild	304M6	*Mj* I-8

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Table S1, end.

Population ID	Habitat	Isolate	63R type
305	Wild	305M1	*Mj* II-12
305	Wild	305M2	*Mj* II-12
305	Wild	305M3	*Mj* I-10(9)
305	Wild	305M4	*Mj* II-12
305	Wild	305M5	*Mj* I-11b
305	Wild	305M6	*Mj* I-11
305	Wild	305M7	*Mj* II-12
305	Wild	305M8	*Mj* I-11b
305	Wild	305M9	*Mj* II-12
305	Wild	305M10	*Mj* II-13
308	Cultivated	308M1	*Mj* I-9(10)
308	Cultivated	308M2/3	*Mj* I-9
308	Cultivated	308M4	*Mj* I-9
308	Cultivated	308M5	*Mj* I-9
308	Cultivated	308M6	*Mj* I-9
308	Cultivated	308M7	*Mj* II-10
308	Cultivated	308M8	*Mj* I-9
308	Cultivated	308M9	*Mj* I-9
309	Cultivated	309M2	*Mj* I-8
309	Cultivated	309M3	*Mj* I-8
309	Cultivated	309M4	*Mj* I-8
309	Cultivated	309M5/6	*Mj* I-8
309	Cultivated	309M7	*Mj* I-8
383	Cultivated	383M2	*Mj* I-10
383	Cultivated	383M3	*Mj* I-10
383	Cultivated	383M4	*Mj* I-10
383	Cultivated	383M5	*Mj* I-10
383	Cultivated	383M6	*Mj* I-10
410	Cultivated	410M3	*Mj* II-13(14)
410	Cultivated	410M9	*Mj* II-14(11)
410	Cultivated	410M10	*Mj* I-9
410	Cultivated	410M12	*Mj* I-9
410	Cultivated	410M13	*Mj* II-9
410	Cultivated	410M14	*Mj* II-14

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Table S2. Mitotype (63R type) occurrence in individuals isolated from cultivated and wild olives. For individuals with heteroplasmy, we only considered the most abundant sequence type (see Table S1). A sequence of each mitotype has been deposited in GenBank.

Mitotype	GenBank no	Cultivated	Wild
Mj I-7	LS974735	5	-
Mj I-8	LS974736	7	12
Mj I-9	LS974737	14	1
Mj I-10	LS974738	5	3
Mj I-11	LS974739	-	1
Mj I-11b	LS974740	-	2
Mj I-12	LS974741	5	1
Mj I-14	LS974742	1	1
Mj II-8	LS974743	7	3
Mj II-9	LS974744	6	3
Mj II-10	LS974745	6	3
Mj II-11	LS974746	1	-
Mj II-12	LS974747	1	5
Mj II-13	LS974748	12	2
Mj II-14	LS974749	3	-
Mj III-8	LS974750	3	-
Mj III-10	LS974751	2	-
Mj III-11	LS974752	2	-
Mj IV-7	LS974753	2	-

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Table S3. Quantitative components of variance for three phenotypic traits: A) stylet size, B) neck width and C) body width. These parameters (σ^2_{Fam}, σ^2_{Ind}, H^2, CV_G) were estimated per population (see text for details).

A) Style size

Population	σ^2_{Fam}	σ^2_{Ind}	H^2	CV_G
252	1.743E-01	9.845E-02	6.391E-01	3.361E-02
253	2.161E-03	3.793E-01	5.665E-03	3.800E-03
258	2.626E-01	1.829E-01	5.894E-01	3.976E-02
259	1.200E-02	2.618E-01	4.384E-02	8.385E-03
260	7.683E-02	1.272E-01	3.766E-01	2.120E-02
261	1.274E-01	2.156E-01	3.714E-01	2.850E-02
262	8.963E-02	2.677E-01	2.508E-01	2.336E-02
285	2.920E-02	2.193E-01	1.175E-01	1.295E-02
308	1.120E-01	3.754E-01	2.297E-01	2.645E-02
309	2.649E-02	9.900E-02	2.111E-01	1.249E-02
383	2.678E-02	7.230E-02	2.703E-01	1.248E-02
410	4.407E-02	1.385E-01	2.414E-01	1.565E-02
296	1.018E-01	8.845E-02	5.351E-01	2.499E-02
300	9.430E-03	6.826E-02	1.214E-01	7.252E-03
305	1.898E-01	1.253E-01	6.023E-01	3.469E-02
301	3.814E-02	6.358E-02	3.750E-01	1.482E-02
304	7.603E-04	1.204E-01	6.277E-03	2.065E-03

B) Neck width

Population	σ^2_{Fam}	σ^2_{Ind}	H^2	CV_G
252	8.210E-03	6.324E-02	1.149E-01	7.654E-03
253	9.537E-03	1.150E-01	7.656E-02	8.530E-03
258	1.036E-02	4.021E-02	2.049E-01	8.656E-03
259	9.213E-03	5.396E-02	1.458E-01	8.020E-03
260	3.680E-02	1.429E-01	2.047E-01	1.646E-02
261	6.720E-03	5.365E-02	1.113E-01	6.926E-03
262	9.128E-03	8.110E-02	1.012E-01	8.202E-03
285	2.515E-10	2.249E-01	1.118E-09	1.370E-06
308	1.519E-02	2.070E-01	6.839E-02	1.064E-02
309	1.287E-02	4.495E-02	2.226E-01	9.901E-03
383	3.648E-02	1.016E-01	2.642E-01	1.647E-02
410	1.354E-02	8.844E-02	1.328E-01	9.938E-03
296	8.254E-03	7.729E-02	9.649E-02	7.648E-03
300	2.431E-03	7.488E-02	3.144E-02	4.168E-03
305	2.669E-02	1.494E-01	1.516E-01	1.385E-02
301	2.160E-02	1.122E-01	1.615E-01	1.220E-02
304	NA	NA	NA	NA

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Table S3, end.

C) Body width				
Population	σ^2_{Fam}	σ^2_{Ind}	H^2	CV_G
252	6.153E-02	3.070E-01	1.670E-01	1.876E-02
253	3.180E-02	6.486E-01	4.674E-02	1.370E-02
258	6.941E-02	1.780E-01	2.806E-01	1.984E-02
259	6.873E-02	1.933E-01	2.623E-01	1.995E-02
260	1.870E-01	3.367E-01	3.571E-01	3.354E-02
261	1.024E-01	2.885E-01	2.620E-01	2.411E-02
262	5.616E-02	4.498E-01	1.110E-01	1.814E-02
285	9.742E-02	7.385E-01	1.165E-01	2.403E-02
308	7.215E-02	4.622E-01	1.350E-01	2.039E-02
309	3.503E-02	1.822E-01	1.613E-01	1.452E-02
383	2.591E-01	5.631E-01	3.151E-01	3.884E-02
410	4.781E-10	7.864E-01	6.079E-10	1.666E-06
296	2.232E-02	2.121E-01	9.520E-02	1.094E-02
300	7.017E-03	1.793E-01	3.767E-02	6.260E-03
305	6.931E-02	3.253E-01	1.756E-01	1.963E-02
301	2.519E-02	3.006E-01	7.732E-02	1.153E-02
304	5.214E-02	5.724E-01	8.348E-02	1.709E-02

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Table S4. F_{ST} values among habitats and between populations of *Meloidogyne javanica* when considering only high-density cultivated and wild populations (2 x 5 populations). This analysis was done either with mtDNA haplotypes or haplogroups.

mtDNA data	Between habitats	Between populations
Haplogroups	-0.0143	0.4703
Haplotypes	-0.0205	0.4237

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Table S5. Effect of habitat (cultivated or wild), population, and family (nested within population) on three life-history traits of *Meloidogyne javanica* sampled in high-density cultivated (C) or wild (W) olive stands. Standard deviation of each value is given in parentheses. Significance of effects was tested by a hierarchical ANOVA: *p*-values are coded as follow: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and NS means non-significant ($p > 0.05$). (An effect of 'Family' on stylet size was marginally significant; $p = 0.06$).

Trait	C	W	Habitat	Population	Family
Stylet size	13.07 (0.52)	13.05 (0.52)	**NS**	**	**NS**
Neck width	11.72 (0.37)	11.86 (0.33)	***	***	***
Body width	13.12(0.74)	13.51 (0.61)	***	*	***

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Table S6. REML variance components for three quantitative traits measured on *Meloidogyne javanica* populations sampled in high-density cultivated and wild habitats. For each trait, we report the variance between habitats (σ^2_{Hab}), between populations within habitat (σ^2_{Pop}), between families within populations (σ^2_{Fam}) and between individuals within families (σ^2_{Ind}). We also assessed the quantitative differentiation between habitats (Q_{ST}) of each trait.

Trait	σ^2_{Hab}	σ^2_{Pop}	σ^2_{Fam}	σ^2_{Ind}	Q_{ST}
Stylet size	8E-09	0.069	0.074	0.133	0.000
Neck width	0.011	0.018	0.014	0.100	0.268
Body width	0.069	0.012	0.070	0.380	0.456

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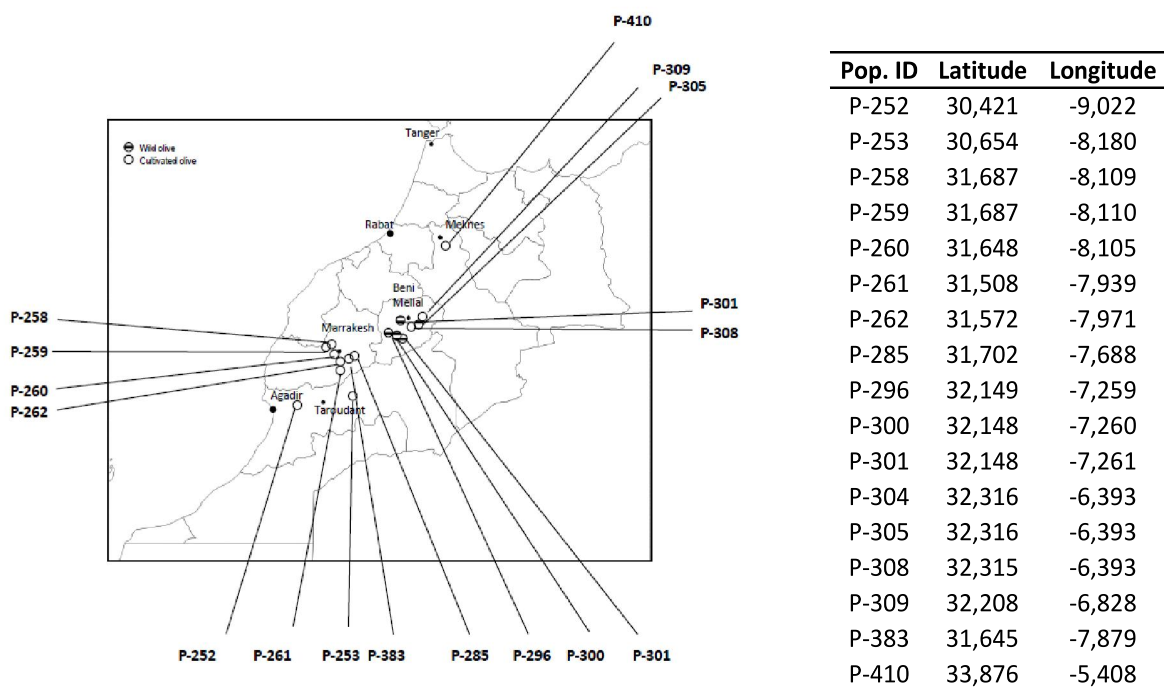

Pop. ID	Latitude	Longitude
P-252	30,421	-9,022
P-253	30,654	-8,180
P-258	31,687	-8,109
P-259	31,687	-8,110
P-260	31,648	-8,105
P-261	31,508	-7,939
P-262	31,572	-7,971
P-285	31,702	-7,688
P-296	32,149	-7,259
P-300	32,148	-7,260
P-301	32,148	-7,261
P-304	32,316	-6,393
P-305	32,316	-6,393
P-308	32,315	-6,393
P-309	32,208	-6,828
P-383	31,645	-7,879
P-410	33,876	-5,408

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Figure S1. Distribution of *Meloidogyne javanica* populations sampled in Morocco (for more details, see Ali *et al.*, 2017. *BMC Ecol.* **17:** 4). Seventeen locations were sampled in 2012. Cultivated and wild stands are represented by white and stripped circles, respectively. GPS coordinates of populations are given on the right.

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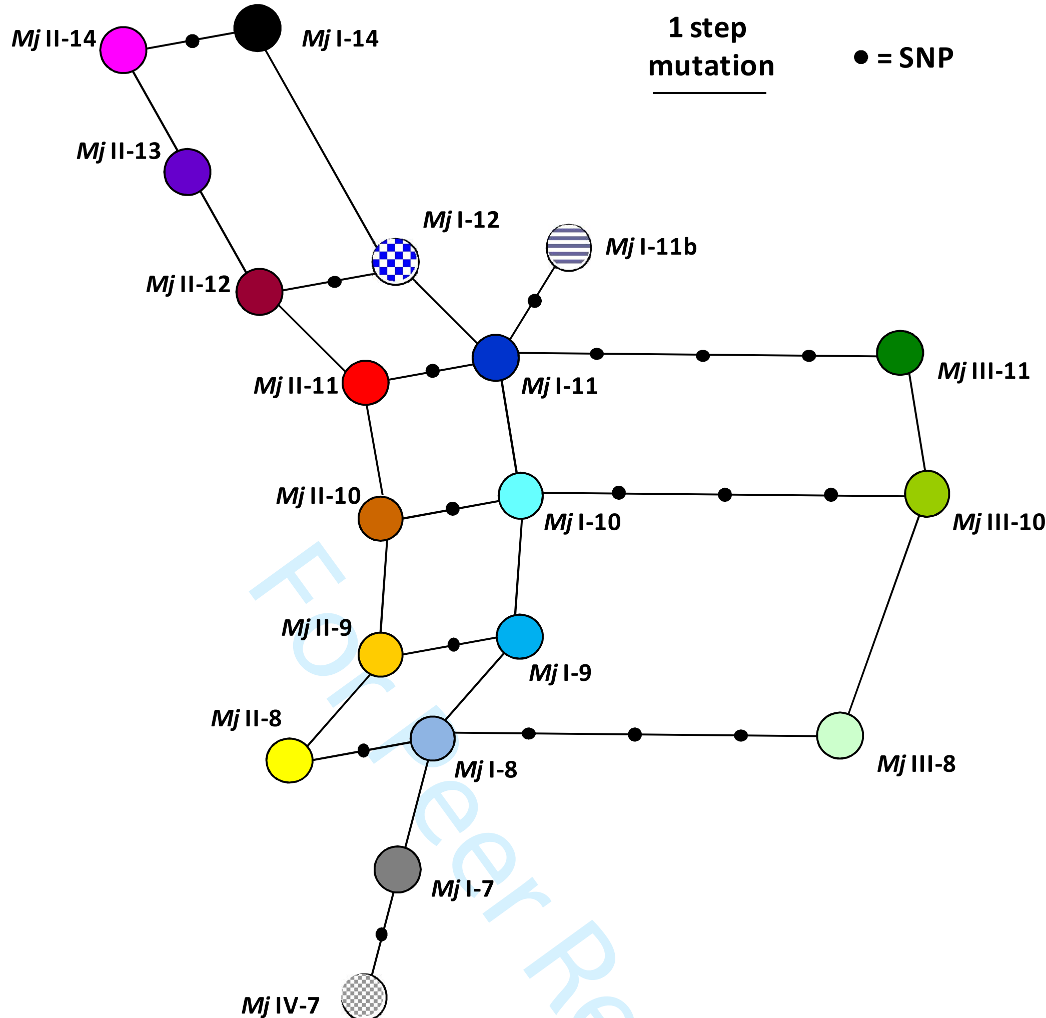

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Figure S2. Mitochondrial haplotype reduced-median network (Bandelt *et al.*, 1999. *Mol. Biol. Evol.* **16:** 37-48). Haplotypes were defined based on five single nucleotide polymorphisms (SNPs) and the number of repeats in a minisatellite in the 63R locus. Nineteen haplotypes were distinguished among isolates (Table S2). The number of minisatellite repeats was coded (from to 7 to 14) as a single multi-state character (considering a stepwise mutation model). The unresolved topology (reticulation) of this network has to be interpreted with caution, but it likely results from homoplasy at the minisatellite motif [i.e., the 63R variants are mostly shared among the three most frequent lineages (defined on SNPs; Fig. 3)].

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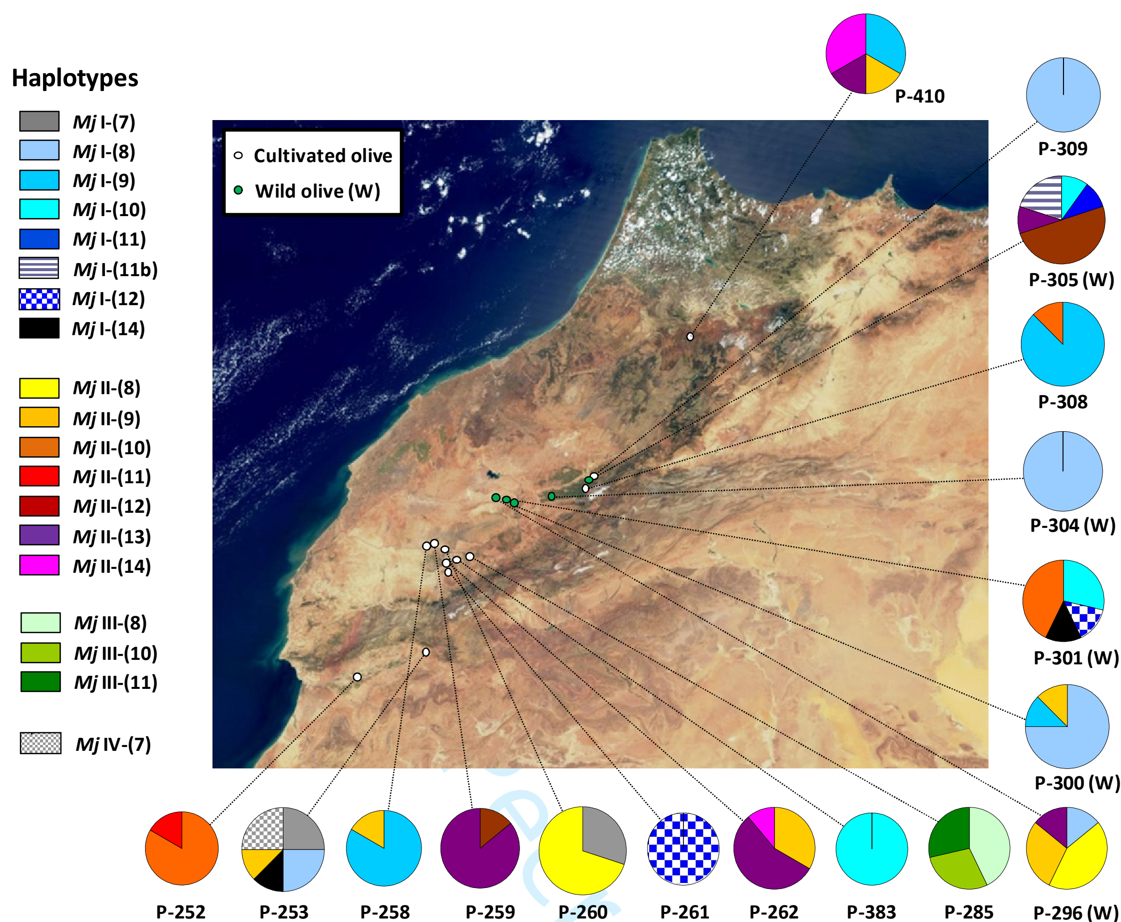

Figure S3. Geographic distribution of mitochondrial haplotypes of *Meloidogyne javanica* in wild and cultivated olive habitats in Morocco (17 populations with five to ten isolates per location). Size of pie charts is proportional to the number of isolates analyzed per location.

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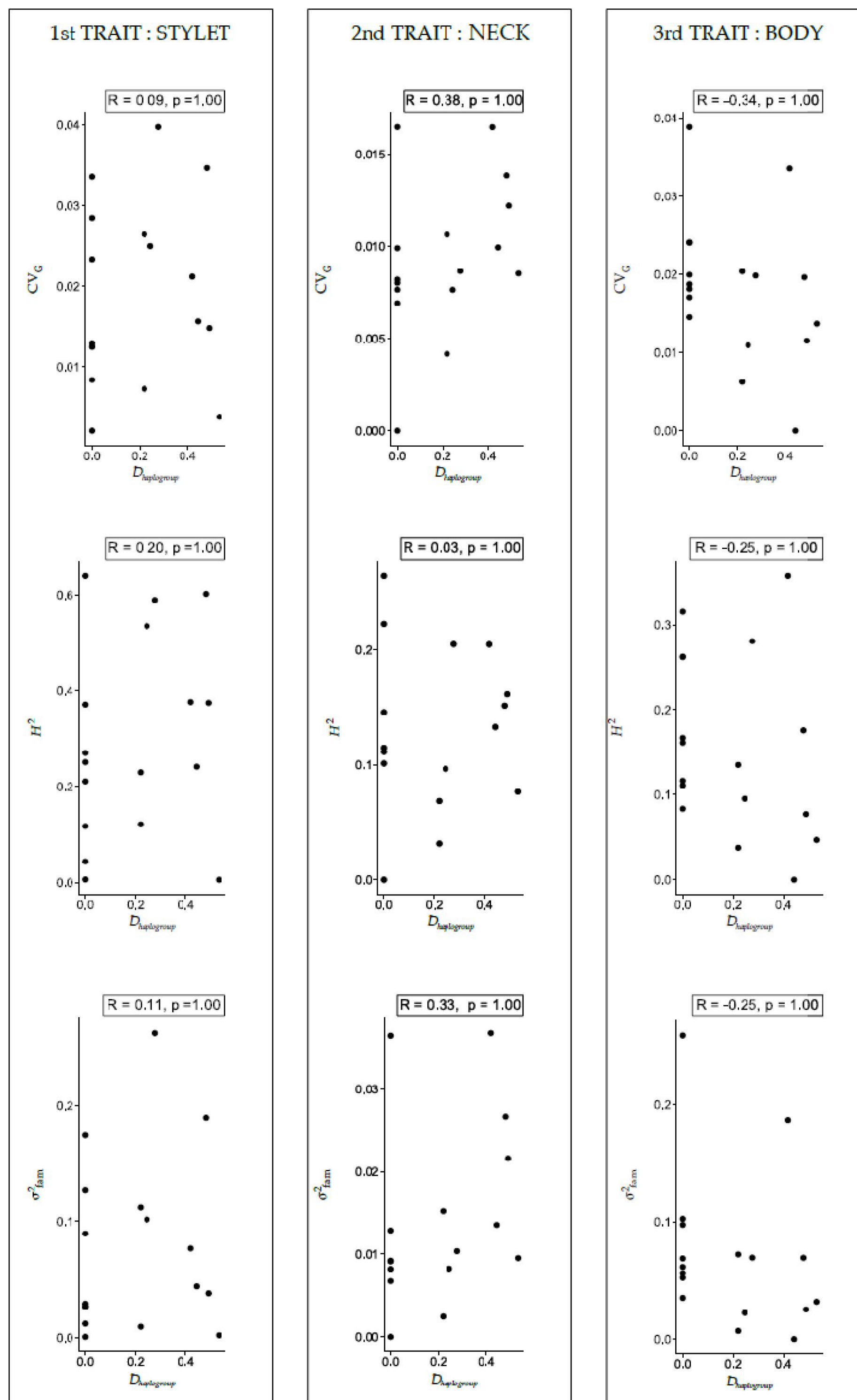

Figure S4. Correlation plot between haplogroup diversity ($D_{haplogroup}$) and quantitative phenotypic diversity calculated for the stylet size (A), neck width (B) and body width (C). Each phenotypic parameter [coefficient of genetic variation (CV_G); heritability (H^2); and family genetic variance (σ^2_{Fam})] is presented on a line. Each point represents a population. For each graph, the Pearson correlation coefficient (R) is given, as well as the *p*-value associated corrected for multiple comparisons (9) using the Bonferroni method.

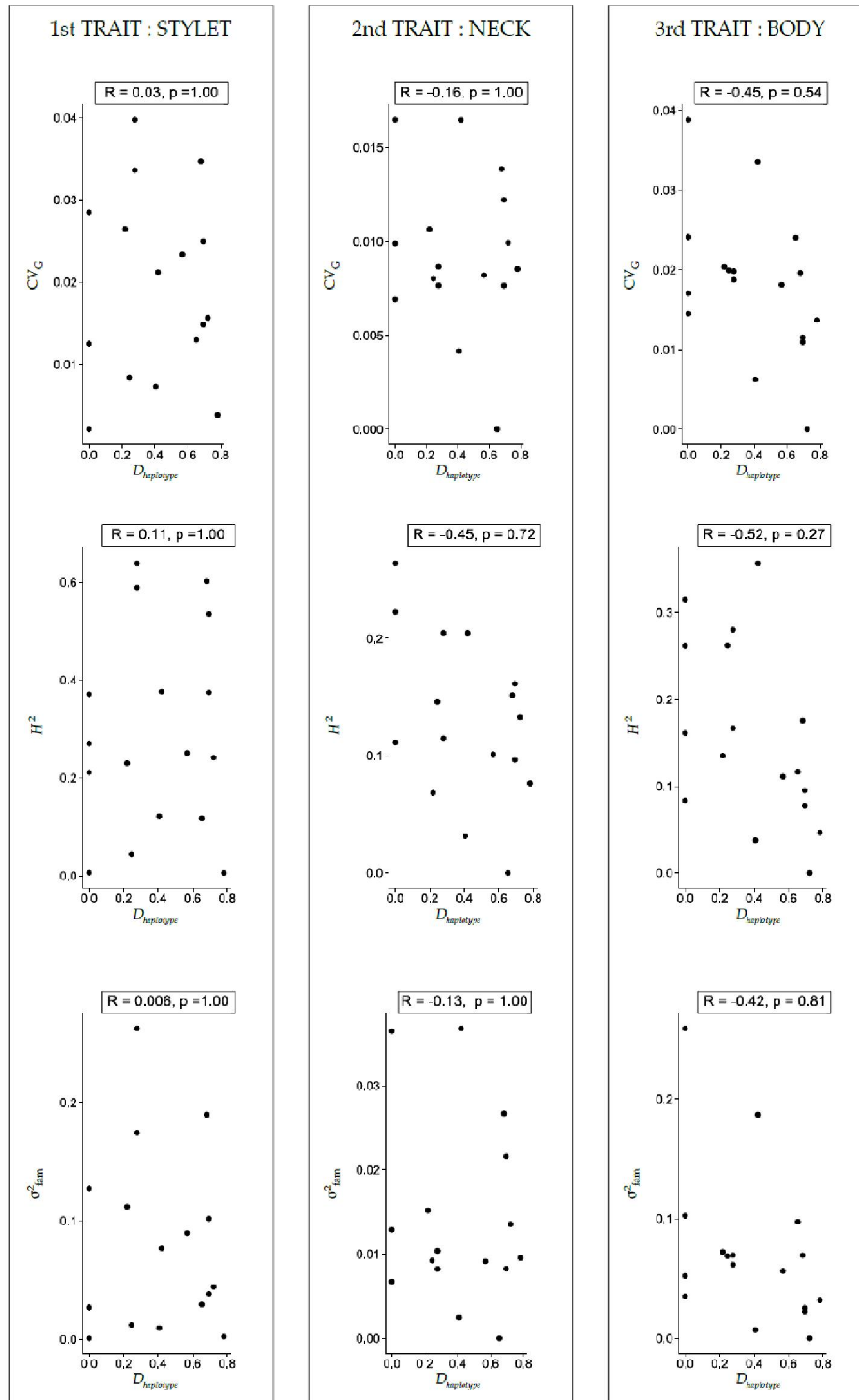

Figure S5. Correlation plot between haplotype diversity ($D_{haplotype}$) and quantitative phenotypic diversity calculated for the stylet size (A), neck width (B) and body width (C). Each phenotypic parameter [coefficient of genetic variation (CV_G); heritability (H^2); and family genetic variance (σ^2_{Fam})] is presented on a line. Each point represents a population. For each graph, the Pearson correlation coefficient (R) is given, as well as the *p*-value associated corrected for multiple comparisons (9) using the Bonferroni method.