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Steinernema feltiae Intraspecific Variability: Infection Dynamics and Sex-Ratio

Raquel Campos-Herrera,[1] Carmen Gutiérrez[1]

Abstract: Entomopathogenic nematodes (EPNs) from the Heterorhabditidae and Steinernematidae families are well-known biocontrol agents against numerous insect pests. The infective juveniles (IJs) are naturally occurring in the soil and their success in locating and penetrating the host will be affected by extrinsic/intrinsic factors that modulate their foraging behavior. Characterizing key traits in the infection dynamics of EPNs is critical for establishing differentiating species abilities to complete their life cycles and hence, their long-term persistence, in different habitats. We hypothesized that phenotypic variation in traits related to infection dynamics might occur in populations belonging to the same species. To assess these intraspecific differences, we evaluated the infection dynamics of 14 populations of *Steinernema feltiae* in two experiments measuring penetration and migration in sand column. Intraspecific variability was observed in the percentage larval mortality, time to kill the insect, penetration rate, and sex-ratio in both experiments ($P < 0.01$). Larval mortality and nematode penetration percentage were lower in migration experiments than in penetration ones in most of the cases. The sex-ratio was significantly biased toward female-development dominance ($P < 0.05$). When the populations were grouped by habitat of recovery (natural areas, crop edge, and agricultural groves), nematodes isolated in natural areas exhibited less larval mortality and penetration rates than those from some types of agricultural associated soils, suggesting a possible effect of the habitat on the phenotypic plasticity. This study reinforces the importance of considering intraspecific variability when general biological and ecological questions are addressed using EPNs.

Key words: entomopathogenic nematode, female-biased, migration assay, penetration assay, sex ratio, *Steinernema feltiae*.

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae are considered excellent biocontrol organisms against numerous insect pests worldwide (Georgis et al., 2006; Kaya et al., 2006). These nematodes are used against pests, primary in horticultural orchards, perennial plantations, and greenhouses cultivation (Kaya et al., 2006; Dolinski et al., 2012). Entomopathogenic nematodes kill the host because of the concomitant activity of a mutualistic enteric γ–Proteobacteria bacteria, *Xenorhabdus* spp. for the nematodes *Steinernema* spp. and *Photorhabdus* spp. for *Heterorhabditis* spp. The bacteria are carried and maintained by the EPN free-living stage, the infective juvenile (IJ) (Boemare, 2002; Sugar et al., 2012). Once the nematodes locate the insect, they actively penetrate the body cavity and release the bacteria in the hemocoel thereby killing the insect, generally in a short time (Boemare, 2002). The digested insect tissues serve as medium for the nematode and bacterial development, and several generations are produced inside the cadaver. When the resources are depleted and excretion products become limiting, a new cohort of IJs is developed, which acquire bacteria and emerge in search of new hosts (Adams and Nguyen, 2002; Boemare, 2002).

The early studies of EPNs focused on more applied questions because these nematodes are excellent biological control agents (see reviews by Georgis et al., 2006; Kaya et al., 2006; Shapiro-Ilan et al., 2006, 2012a). More recently, the number of basic studies in which EPNs are used as models to address general biological, ecological, and evolutionary questions has significantly increased (Stock and Goodrich-Blair, 2008; Campos-Herrera et al., 2012; Griffin, 2012; Kaplan et al., 2012). From an ecological perspective, foraging strategies and motility are important variables in population dynamic models (Campos-Herrera et al., 2012). These variables are influenced by abiotic and biotic environmental factors (Lewis et al., 2006; Stuart et al., 2006) and by intrinsic nematode traits. Campbell and Gaugler (1997) described two foraging tendencies that are species specific. Nematodes described as "cruisers" are active and move in the soil profile following different chemical and physical signals whereas "ambushers" remain near or on the soil surface, and are characterized by less active movement, but rather attacking insects moving nearby. There is a continuum between both extremes, with nematodes sharing properties of both foraging activities, as in the case of the EPN species *S. feltiae* (Campbell et al., 2003). More recently, Ennis et al. (2010) demonstrated that *S. carpocapse*, typically an ambusher EPN, may behave as a cruiser in the presence of twigs and, therefore, exhibits a higher level of complexity in response to varying environmental factors. Additional factors that can interact with the nematode foraging activity include molecules secreted by plants and insects cues (Rasmann et al., 2005; Ali et al., 2012; Dillman et al., 2012); and even electric fields (Shapiro-Ilan et al., 2009, 2012b).

Early studies on the penetration dynamics of steinernematids suggested the "male-colonizer IJs" hypothesis, suggesting that males are the pioneer sex involved in the search and infection dynamics process (Grewal et al., 1993). However, this idea was not supported by subsequent studies, where EPN species, IJ age, production media, among other factors, drove the proportion to a "non-biased-penetration" by sex (Stuart et al., 1998) and in some cases a "female-biased" proportion (Fujimoto et al., 2007; Alsaiyah et al., 2009).

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[1]Departamento de Contaminación Ambiental, Instituto de Ciencias Agrarias, CSIC, Serrano 115 Dpdo, Madrid, Spain.
E-mail: raquel.campos@ica.csic.es
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The sex in EPN is determinate and, therefore, is already defined in the IJ steinernematid stage, before the maturation process inside the host (Bohan and Hominick, 1997). Alsaiyah et al. (2009) studied the infection dynamics and sex-ratio in five steinernematids species, including *S. feltiae*. They observed that male and female IJs differ in their infection behavior and indicated that the proportion of males establishing in insects was dependent on the experimental conditions. When those authors evaluated the sex ratio in the population using an in vitro assay (i.e., IJs developing to adults in hanging drops of insect haemolymph), the population was female-biased and suggested that this higher proportion of females could be the optimal sex ratio. However, it is unknown if these female-bias occur in the same manner in populations from the same species with different life history and habitat adaptations. Therefore, from an ecological perspective, it results interesting to evaluate the intraspecific variability in the penetration rate and relative contribution of each sex on the process.

The intraspecific variability in critical traits associated with the infection dynamics and ability to complete the life cycle could result from different environmental pressures. Several isolates of *S. feltiae* from various habitats in La Rioja (Northern Spain) exhibited intraspecific variability in reproduction, virulence against the agricultural pests *Ceratitis capitata* (Diptera: Tephritidae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae), and the physiology and molecular profile of their associated bacteria (Campos-Herrera et al., 2007, 2008, 2009; Campos-Herrera and Gutiérrez, 2009). Considering previous studies, these well-characterized nematodes are excellent candidates to study the intraspecific variability of traits contributing to host infection. Our hypothesis was that patterns of infection-related traits will differ among different populations of genetic variability because of their adaptation to different habitats. This study measured the infection dynamics of 14 native populations of *S. feltiae* isolated from different localities and habitats in (i) a penetration assay, which describes the ability of the EPN to penetrate and kill the insect given a continuous contact between the nematodes and the host; and (ii) a migration assay, evaluating the movement of the IJs in a sand column. In both cases, the numbers of males and females were determined to assess the possibility of a sex bias.

Materials and Methods

Entomopathogenic nematode populations: Fourteen native isolates of *S. feltiae* were recovered from soil samples in natural areas, crop field edges, and agricultural areas in La Rioja (Northern Spain) by using the *Galleria mellonella*–insect baiting technique (Bedding and Akhurst, 1975) (Table 1). Sites, even those in the same locality, were separated by several kilometers. Differences in the environmental and soil characteristics of the studied area were previously described by Campos-Herrera et al. (2007, 2008). Species identification was performed by using morphological and molecular tools (Campos-Herrera et al., 2007, 2008; Nguyen, 2007). Studies of the symbiotic bacteria allowed their identification as *Xenorhabdus bovienii* and their characterization established two different genotypes (Campos-Herrera et al., 2009). The IJs were produced by placing six *Galleria mellonella* (Lepidoptera: Pyralidae) larvae per petri dish (5.5-cm diam., n = 3 per EPN population), lined with filter paper and moistened with a suspension of nematodes adjusted to provide a final concentration of 100 IJs/cm^2 petri surface. The emerging nematodes were maintained in a water suspension at 10°C for 1 to 2 wk before use. New nematode cultures were produced for each experiment/repetition to test different IJ cohorts. IJ concentrations were adjusted by volumetric dilutions in mQ-water (Milli-Q Water System, Millipore S.A., Molsheim, France) following the method described by Glazer and Lewis (2000).

Table 1. Environmental variables associated with the locality where the 14 *Steinernema feltiae* populations were isolated in La Rioja (Northern Spain).

Type of habitat	Locality name	Population code[a]	Altitude (masl)[b]	pH	Clay content (%)
Natural area (N)	Ruedas de Ocón	37	812	5.3	55
	Viniegra de Arriba	58	1,569	7.9	40
	Valdezcaray	66	1,754	6.6	53
	Bañares	BZ	617	5.5	52
Crop edge (CE)	Yerga	17	650	7.7	49
	Préjamo	23	699	7.9	70
	Pipaona	38	695	5.7	54
	Villarejo	63	785	8.3	40
	Camprovín	75	686	7.8	52
	Santo Domingo de la Calzada	91	639	7.1	58
	Santo Domingo de la Calzada	100	651	6.5	37
Perennial crop (PC)	Bañares	BV	599	5.2	31
	Leiva	LV	625	6.9	27
	Leiva	LF	598	7.6	26

[a] Population code corresponds with the previous reports. For original data and more detailed information, see Campos-Herrera et al. (2007, 2008).
[b] masl = meters above sea level.

Entomopathogenic nematodes infection dynamics and sex ratio assessment: The infection dynamics and IJs penetrant sex-ratio of 14 *S. feltiae* EPN isolates were assessed in two experiments using *G. mellonella* as host (i) penetration and (ii) migration in sand column (Glazer and Lewis, 2000; Alsaiyah et al., 2009). The penetration experiment was performed using a modified procedure from Bedding et al. (1983) with multiplate recipients (25-well plates, 4-cm^2/well) (Sterilin Hounslow, Middlesex, UK) with a lid that adjusted and isolated each of the wells independently. Each well on the 25-well plates were filled with a thin layer (0.8 g/well) of sterile sand (1.6 to 0.16 mm particle size) and moistened with 50 μL mQ-water as described in Campos-Herrera and Gutiérrez (2009). Then, for each nematode population, 200 IJs were applied per well in a final volume of 100 μL in mQ-water suspension. Controls were prepared following the same procedures but adding 100 μL of mQ-water in each well. One larva of *G. mellonella* was placed per well (controls and EPN treatments), and finally covered with the lid, avoiding cross-contamination among wells. The plates were incubated in a growth chamber (22 ± 1°C, 55 ± 5% relative humidity, L16:D8 photoperiod). Larval mortality was assessed daily during a 7-d period. All cadavers were rinsed in tap water and kept individually in new petri dishes with a clean and moistened filter paper until dissection. The number of nematodes inside each cadaver and their sex were established by cadaver dissection under a stereoscopic microscope 2 to 3 d after insect death. All bioassays were repeated twice per population (n = 25, two independent trials per populations), using new and fresh nematodes each time, and with their corresponding control treatments.

The migration experiment was performed using 15 plastic tubes (35-mm-diam. × 40-mm) per nematode population and control. For each nematode population, 100 IJs were applied per tube in a final volume of 100 μL in mQ-water suspension; tubes for control treatment were prepared in the same manner but adding 100 μL of mQ-water on each. Then, all the tubes were filled with sterile moistened sand (8% v/w) and a larva was added at the end of the tube (opposite to the nematodes), closing with a cap to limit and ensure the movement. Inverted tubes were incubated for 24 h in a growth chamber as previously described above. The larvae were then rinsed in tap water and placed in separate petri dishes lined with filter paper and incubated for another 72 h. Larval mortality was checked daily and cadavers were processed as described in the penetration experiment. All bioassays were repeated twice per population (n = 15, two independent trials per populations), with their corresponding control treatments.

Statistical analysis: The variables considered in both assays were (i) percentage larval mortality, (ii) time to larva death, (iii) nematode penetration percentage, and (iv) sex-ratio, measured as the proportion of males and females inside each host. Only the data corresponding to percentages (mortality and penetration) were transformed before statistical analysis, by using arc sin transformation (Campos-Herrera and Gutiérrez, 2009). The intraspecific variability of the response variables was evaluated by one-way analysis of variance (ANOVA) (n = 14), using Tukey's test to assess mean differences ($P < 0.05$). Differences on the numbers of male and female observed inside the cadaver individually for each population and for the whole experiment (using the populations as replicates) were assessed by paired *t*-tests ($P < 0.05$). We employed the nonparametric Kruskal-Wallis test ($P < 0.05$) to describe the possible influence of the habitat type (natural areas, crop edge, and perennial crop) in all these variables described above, using the populations as replicates per habitat type. Additionally, the correlation among the mean values for mortality, penetration, days to larval death and environmental variables shown in Table 1 were analyzed by the nonparametric Rho of Spearman (exact test, $P < 0.05$). Results are presented as mean ± SEM of untransformed variables. These statistical analyses were performed with the software SPSS 20.0 (SPSS Inc., Chicago, IL).

Results

Intraspecific variability in penetration assay: Intraspecific differences among the 14 *S. feltiae* populations were detected for all the variables studied ($P < 0.01$; Fig. 1). Most of the populations produced similar larval mortality, with only two statistically significant extremes, populations 17 and 66 (below 50%) compared with population 100 that registered 97.4% (Fig. 1A). Similarly, intraspecific differences among populations were observed in the number of days to kill the larva ($P = 0.002$), which was slightly above 2 d for 10 out of the 14 populations (Fig. 1B). Population 17 killed (1.2 ± 1.5 d) was significantly quicker than Populations 63, LV and LFE (Fig. 1B). The nematode penetration rate was variable ($P < 0.001$, Fig. 1C), ranging from 3.7% to 4.7% (populations 66 and 23) to above 30% (population 63). Generally, females predominated in the cadavers ($P < 0.001$, Fig. 1D). Only two populations did not differ in the number of IJs-developed to males and females that penetrated the host (population 58 and 66).

Intraspecific variability in migration assay: Differences in larval mortality among populations were also observed ($P < 0.001$) (Fig. 2A). The significant differences were only among the most extreme effects of 20% mortality (population 58) and above 90% (populations 37 and LV). The number of days to kill the larva by was above 2 d in all cases except for population 58 from natural areas that required 4 d (Fig. 2B). The maximum nematode penetration percentage recorded in this assay was 6.8 ± 4.2%, registered by population LV (Fig. 2C),

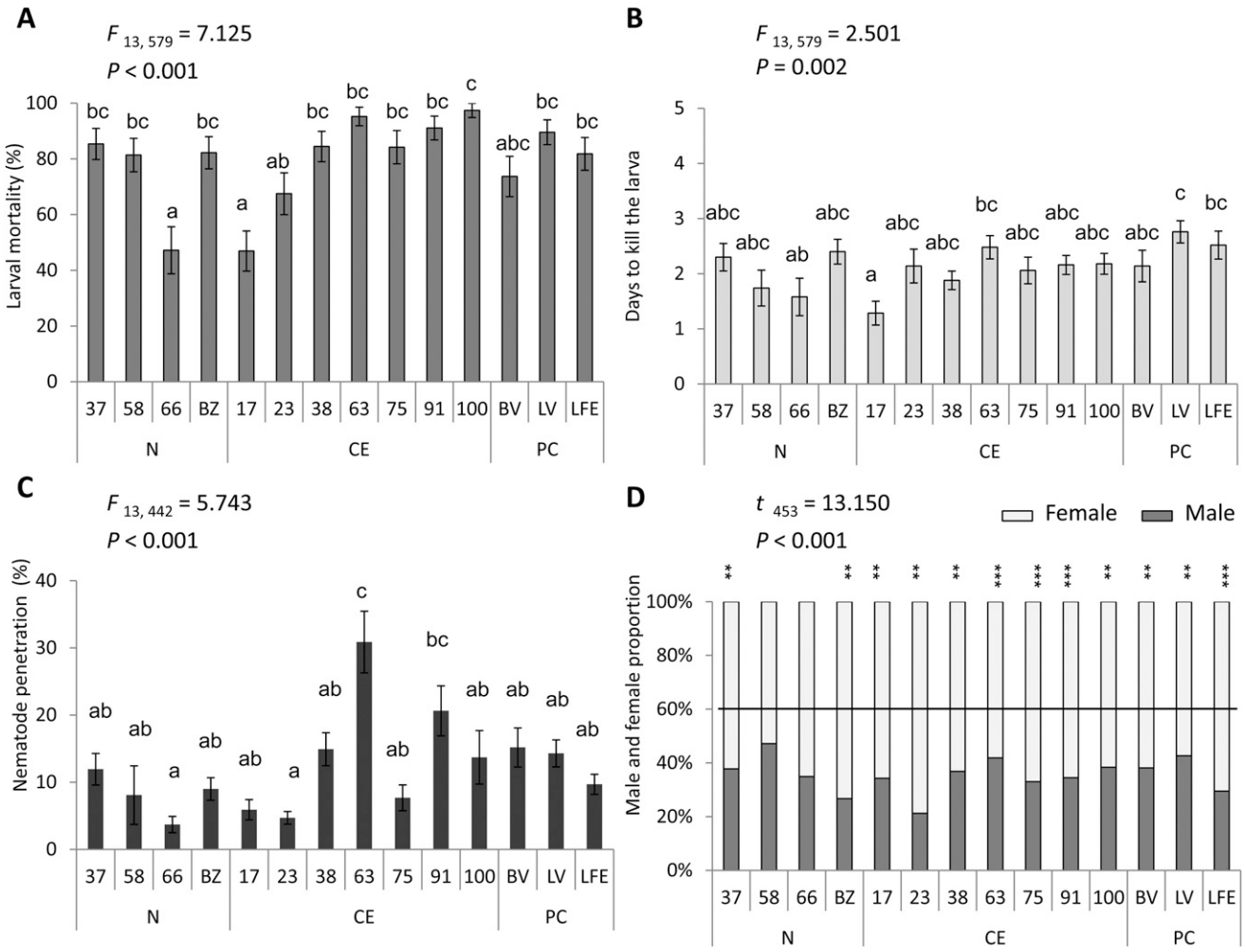

FIG. 1. Intraspecific variability in infection dynamics derived from the penetration experiments for 14 populations of *Steinernema feltiae* (N: natural areas; CE: crop edge; PC: perennial crop). A. Mortality percentage. B. Days to kill the insect. C. Nematode penetration percentage. D. IJ development to male and female proportion inside the cadaver. Means that were significantly different in one-way ANOVA analysis and Tukey's test were represented by different small letters ($P < 0.05$). Differences on the numbers of male and female observed in the cadaver on each population and for the whole experiment were assessed by a paired *t*-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Data are shown as means ± SEM of the untransformed data.

whereas 11 out 14 populations recorded penetration significantly lower than this maximum (< 3.3%). In agreement with the observations in the penetration assay, there was also a significant bias to female as nematode invader ($P < 0.001$; Fig. 2D). In this case, only the number of males and females in population BZ did not differ inside the cadaver.

Infection dynamics and isolation habitat: In general, populations from natural areas exhibited lower larval mortality percentages (Fig. 3A), although these differences were only statistically significant in the migration assay ($P < 0.001$). Whereas the populations isolated from natural areas killed the insect in a shorter time in the penetration assay, the opposite trend was observed in the migration experiment (Fig. 3B). Lower nematode penetration percentage was registered by populations isolated from natural areas in both experiments (Fig. 3C). The sex-ratio was significantly biased to the female dominance in both experiments and for the three habitats considered in the analysis (Fig. 3D).

The altitude and clay content associated with nematodes isolates showed a positive trend ($P = 0.076$), similarly to altitude and pH with respect the number of days to kill the larva in the migration experiment ($P = 0.073$, and $P = 0.054$, respectively) (Table 2). Natural areas were located in high elevations whereas both the crop edge and perennial crop isolates were associated with the valley. The managed area, perennial crop, had less than the half of the clay content than the unmanaged areas (natural and crop edge) (see Table 1). Then, it is plausible that native populations from natural areas tended to be slower on killing the larva, and reduce the movement as required in the migration experiment. Moreover, the mortality percentage recorded in the penetration experiment was positively correlated with the mortality in the migration bioassay ($P = 0.046$) as well as the number of days recorded to

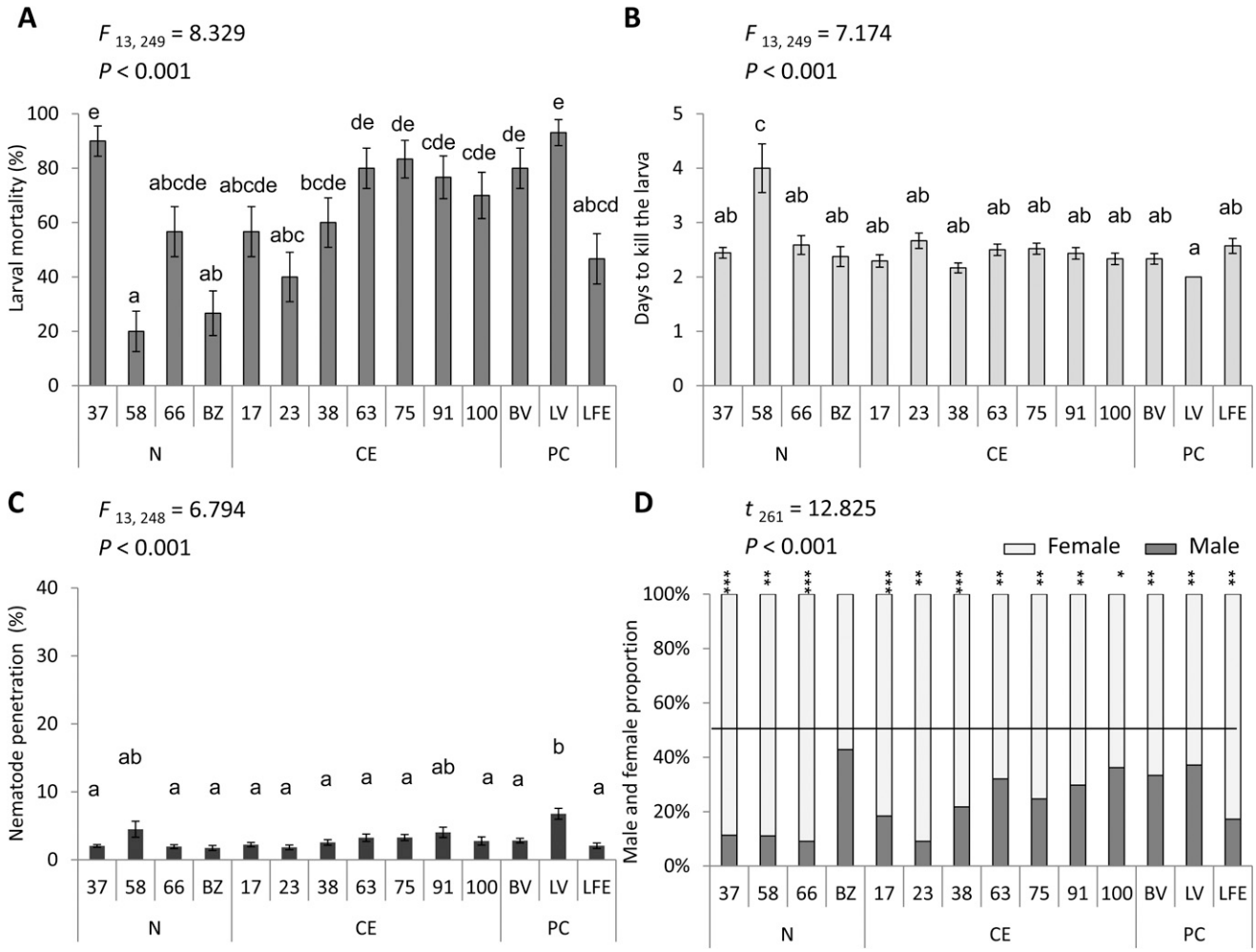

FIG. 2. Intraspecific variability in infection dynamics derived from the migration experiments for 14 populations of *Steinernema feltiae* (N: natural areas; CE: crop edge; PC: perennial crop). A. Mortality percentage. B. Days to kill the insect. C. Nematode penetration percentage. D. IJ development to male and female proportion inside the cadaver. Means that were significantly different in one-way ANOVA analysis and Tukey's test were represented by different small letters ($P < 0.05$). Differences on the numbers of male and female observed in the cadaver on each population and for the whole experiment were assessed by a paired t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Data are shown as means ± SEM of the untransformed data.

kill the larvae ($P = 0.007$) (Table 2). In regard to the penetration ability recorded in the penetration experiment was positively associated with the produced mortality ($P = 0.003$) and negatively with the number of days to kill the larvae ($P = 0.007$) (Table 2). This negative association was also observed between the mortality and the days to kill the larvae ($P = 0.033$) (Table 2).

DISCUSSION

The study of 14 populations of *S. feltiae* isolated in three habitats (natural areas, crop field edges, and agricultural crops) revealed intraspecific variability on variables describing the infection process (larval mortality, time to kill the insect, and penetration rate) and we observed a sex-ratio biased toward a higher female proportion.

Larval mortality and nematode penetration percentage were lower in migration experiments than in penetration ones in most of the cases. The mortality and penetration rates observed in the penetration experiment for populations isolated from agricultural areas (crop edges and perennial crops) were similar to those reported for the native *S. feltiae* Rioja population from the same region (Campos-Herrera et al., 2006) and to those in studies on the intraspecific variability of *S. feltiae* by Gaugler et al. (1989) and Tarasco (1997). However, our results contrasted with rates above 30% reported by Caroli et al. (1996) and Ricci et al. (1996) for other population of *S. feltiae*.

Larval mortality percentage and the nematode penetration percentage were generally lower in the migration bioassay. In general, larval mortality decreased from 79% in penetration to 63% in migration assay, and the nematode penetration was more than fourfold lower in the migration assay. This experiment submits the nematodes to more restrictive conditions, such as lower concentration (2.6 IJs/cm^3 in migration assay

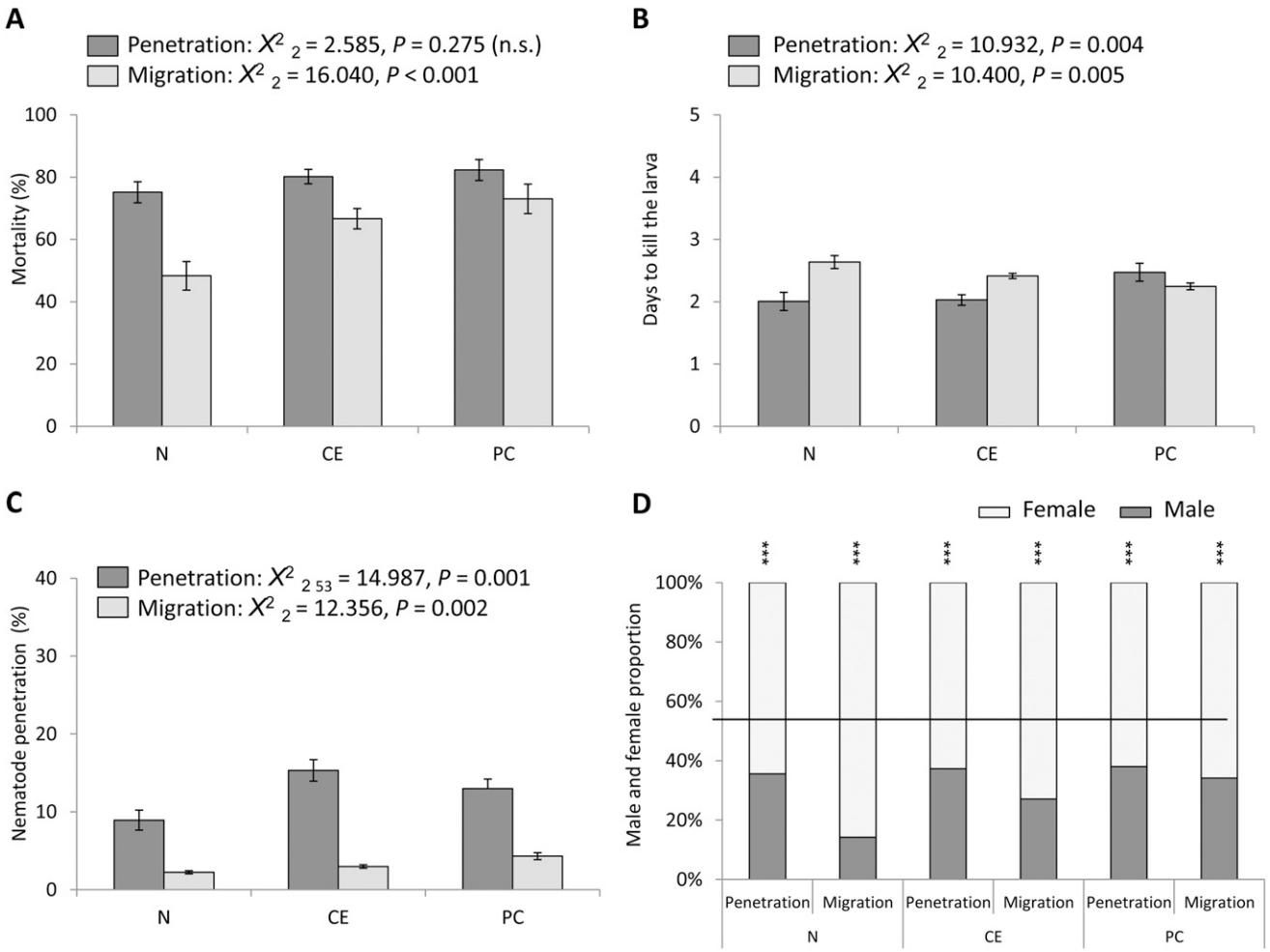

FIG. 3. Intraspecific variability in infection dynamics derived from the penetration and migration experiments based on the origin of isolation (N: natural areas; CE: crop edge; PC: perennial crop). A. Mortality percentage. B. Days to kill the insect. C. Nematode penetration percentage. D. IJ development to male and female proportion inside the cadaver. Means that were significantly different in non parametric Kruskal-Wallis analysis ($P < 0.05$). Differences on the numbers of male and female observed in the cadaver per habitat were assessed by a paired t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Data are shown as means ± SEM of the untransformed data.

versus 50 IJs/cm^2 in penetration assay), requires the active migration of these IJs throughout the sand column to locate the insect and limits the nematodes exposure to the host to 24 h. However, the trends were similarly to those of the penetration experiment. Mortality rates caused by the populations from agricultural-associated areas (crop edges and perennial crops) were in agreement with these found in other studies (Ricci et al., 1996; Glazer and Lewis, 2000; Campos-Herrera et al., 2006), although the nematode penetration rates were 20-fold lower than those of population OBSIII of *S. feltiae* (Glazer and Lewis, 2000). In a recent study of native *S. feltiae* populations isolated in Catalonia, intraspecific differences were also observed in the infection dynamic assessed in a migration assay (Morton and García del Pino, 2009). In that study, most of the *S. feltiae* populations recorded 100% mortality. However, comparison between our results and these reported by Morton and García del Pino (2009) has to be made with caution, since the experimental procedures included longer time of exposure to the nematodes (72 vs. 24 h), higher nematode concentration (1,000 vs. 100 IJ) and longer experimental units (20 vs. 4 cm).

As Bohan and Hominick (1997) described, the sex is already determined in the steinernematid IJ. Therefore, the sex-ratio of penetrant IJs is critical for steinernematids because, with the exception of *S. hermaphrodicum* (Griffin et al., 2001), the species in this genus require at least one female and one male in a host to allow reproduction. Grewal et al. (1993) suggested that the IJ males were more successful in their searching and penetration ability, suggesting the "male-colonizer" hypothesis for steinernematids. However, subsequent studies were not in agreement with this hypothesis, suggesting that the colonizer sex-ratio could be species dependent (Lewis and Gaugler, 1994) or affected by experimental procedures (Stuart et al., 1998; Fujimoto et al., 2007; Alsaiyah et al., 2009). In general, our results on sex-ratio in both experiments were in agreement with previous reports of a tendency for female

TABLE 2. Nonparametric correlation (Rho of Spearman, *P*) among environmental and biological variables (penetration and migration bioassays).

Variables		Altitude masl[a]	Clay content %	pH	Penetration assay			Migration assay		
					Mortality %	Penetration %	Days to kill larva	Mortality %	Penetration %	Days to kill larva
Altitude masl		-	0.489	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.493
			0.076*							0.073*
Clay content %			-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
pH				-	n.s.	n.s.	n.s.	n.s.	n.s.	0.524
										0.054*
Penetration assay	Mortality %				-	0.733	-0.572	0.540	n.s.	n.s.
						0.003***	0.033**	0.046**		
	Penetration %					-	-0.688	n.s.	n.s.	n.s.
							0.007***			
	Days to kill larva						-	n.s.	n.s.	0.682
										0.007***
Migration assay	Mortality %							-	n.s.	n.s.
	Penetration %								-	n.s.
	Days to kill larva									-

[a] masl = meters above sea level.
* $P \leq 0.10$, ** $P \leq 0.05$, *** $P \leq 0.01$.

dominance in the infection process (Tarasco, 1997; Renn, 1998; Campos-Herrera et al., 2006; Fujimoto et al., 2007; Alsaiyah et al., 2009).

The larval mortality rate was higher for populations from agricultural-associated areas in the migration assay, with the same nonsignificant trend for the penetration study, which was in accordance with a higher nematode penetration rate. In the penetration experiment, nematodes from these areas required more time to kill the host, whereas in the migration experiment, these nematodes moved faster than the nematodes from natural areas. Since the clay content was higher in the crop edges and perennial crops (Table 1), it is plausible that nematodes isolated there might be better adapted to migrate in heavy soils, and hence, moved more quickly in the sand substrate of our assay, although this variable did not account for any significant relationship with the biological variables (Table 2). Then, although texture affects EPN movement in the soil (Lewis et al., 2006; Stuart et al., 2006), these isolates may respond differently to many other soil characteristics and insect species (Koppenhöfer and Fuzy, 2006). Our results highlight the importance of including different populations of the same species when biological and ecological characterizations are performed, in order to recover the maximum range of expression of these variables.

Another plausible explanation for the intraspecific variability could be attributed to intraspecific differences in the foraging behavior based on the insect found common to each site. The IJs of *S. feltiae* have been described as behaving as an intermediate activity, into the established continuum between cruisers and ambushers (Campbell and Gaugler, 1997). However, a subsequent theoretical study by Fenton and Rands (2004) showed that the foraging strategy was dependent on the likelihood of contact with the insect, and hence, the cost/profit of the EPN activity was an important component in the total balance. Moreover, experimental results from a recent study have shown that this categorization (cruiser/intermediate/ambusher) was also dependent on the ecological scenarios. Ennis et al. (2010) observed that the same cohort of IJs of *S. carpocapsae*, the most representative EPN ambusher, was able to move using twigs as simulated roots, as "soil route ways." Together with these new observations against the ambusher/cruiser typical searching behavior, we can also link the novel idea of group movement versus individual choices, suggested by El-Borai et al. (2011), for which possible advantages and disadvantages have been summarized by Griffin (2012). Further, it is possible that the movement/migration was not only modulated by host attraction but by more selected chemical attractions such as those recently reported by Choe et al. (2012) and Kaplan et al. (2012), and those induced by plants (Rasmann et al., 2005; Ali et al., 2012).

Several component of infection processes that determine the success of the EPN life cycle were investigated for 14 populations of *S. feltiae*. Understanding key intrinsic and extrinsic factors affecting the infection dynamics can improve our ability to exploit and manage EPNs for biological control (Campos-Herrera et al., 2012; Griffin, 2012). In this study, we have shown how different nematodes from the same species might differ in different traits associated with the infection dynamic, such as the mortality, the time to kill the larva, and the penetration percentage, by using populations isolated in different locations that might represent a possible phenotypic plasticity derived from local adaptation. To better understand population differences, additional studies on the migration activity in various environmental conditions will help us to formulate a more accurate model of migration in the soil profile, and the infection process.

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