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Phenotypic trait changes in laboratory – reared colonies of the maize herbivore, *Diabrotica virgifera virgifera*

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Abstract

The North American and European maize pest *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) was used to assess whether conditions of the natal field, subsequent laboratory rearing, or genetic population origin affect phenotypic traits of fitness, activity, or morphometrics. Standardized laboratory bioassays with large sample sizes revealed that none of the 16 tested traits, except crawling behaviours, appeared consistently stable across all seven tested colonies. Environmental conditions in the natal field of the F_0 generation affected trait averages of the subsequently reared F_1 generation in laboratory in *ca.* 47% of cases, and trait variability in 67% of cases. This was apparent for fitness and morphometrics, but less obvious for activity traits. Early generation laboratory rearing affected trait averages in *ca.* 56% of cases: morphometrics changed; fecundity and egg survival increased from F_1 to F_2. Trait variability increased or decreased in 38% of cases. Laboratory rearing for over more than 190 generations affected the trait averages in 60% of cases, reflected by decreases in flight activity and increases in body size, weight, and fecundity to some extent. It had little effect on trait variability, especially so for morphometric variability. The genetic population origin affected average levels of 55% and variability of 63% of phenotypic traits. A comparison among *D. v. virgifera* studies might be difficult if they use different populations or laboratory colonies. It is advised to consider possible effects of original field conditions, laboratory rearing, and population genetics when planning comparative studies targeting fitness, activity, or morphometric questions regarding *Diabrotica* species.

Keywords: phenotypic traits, fitness, activity, morphometrics, generation number, western corn rootworm, Coleoptera, Chrysomelidae, *Zea mays*

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Introduction

Measures of insect phenotypic traits are used to address a diverse range of basic and applied research questions. For example, phenotypic life history traits and their heritability are often investigated in theoretical insect ecology

(e.g., Spurgeon, 2012) and evolutionary biology (e.g., Hill & Caballero, 1992; Stearns, 1992; Miyatake & Yamagishi, 1999; Khazaeli & Curtsinger, 2010). Behavioural phenotypic traits of pest insects are specifically investigated to better understand questions in applied insect ecology (Huettel, 1976), usually with the goal of improving pest management strategies (Richerson & Cameron, 1974; Prokopy *et al.*, 1975).

Because of their relatively small size, ease of observation, and high rate of reproduction, many studies on insects are conducted under laboratory conditions. Laboratory studies can reduce environmental variation allowing researchers to focus on biological variation within and among populations. Often insects are easily mass-collected from the field and brought into the laboratory at different times for rearing specific colonies, which results in studies on specimens from colonies of varying origins, initial (=founder) population sizes, and generation numbers. A number of studies have shown that working in the laboratory either with field-collected individuals or with long-term mass-reared colonies can both have limitations (Richerson & Cameron, 1974; Prokopy *et al.*, 1975; Bush *et al.*, 1976; Huettel, 1976; Boiler & Chambers, 1977; Chambers, 1977). For example, they might differ in traits due to trans-generational environmental effects originating from the field conditions, also called parental effects from the field (Diamond *et al.*, 2010) or due to geographical distances among populations of origin (Diamantidis *et al.*, 2011). An advantage of using field-collected insects is that they have not been selected for adaptation to certain laboratory conditions (Rossler, 1975). An advantage of laboratory-reared insects is that those trans-generational effects can be standardized to laboratory conditions, i.e., laboratory rearing can eliminate differential responses specific to the environment in the different fields. But, this carries the risk of laboratory adaption, i.e., genetic changes in traits over generations may appear (Rossler, 1975; Miyatake & Yamagishi, 1999; Scannapieco *et al.*, 2009; Spurgeon, 2012).

One of the most economically important agricultural pest insects, the western corn rootworm, *Diabrotica virgifera* ssp. *virgifera* LeConte (Coleoptera: Chrysomelidae) is often studied in the field or using specimens that can be easily collected from the field. However, many studies use beetles from laboratory-reared colonies (Lefko *et al.*, 2008).

Diabrotica v. virgifera is a univoltine maize pest, which overwinters as eggs in the soil (Chiang, 1973; Krysan & Miller, 1986). After maize has germinated, the eggs hatch and the three larval instars feed almost exclusively on maize roots (Moeser & Hibbard, 2005) often causing severe damage leading to plant lodging and extensive yield losses. Adults emerge around flowering stage of maize (Toepfer & Kuhlmann, 2006), and can reduce crop yields through silk feeding, which interferes with maize pollination (Chiang, 1973; Culy *et al.*, 1992).

Diabrotica v. virgifera is hypothesized to have originated in Mexico, where a number of pestiferous *Diabrotica* species occur (Branson & Krysan, 1981; Krysan & Smith, 1987). With the expansion of monoculture maize-growing in the second half of the 20th century, *D. v. virgifera* became a major pest of maize in the USA (Krysan & Miller, 1986; Levine & Oloumi-Sadeghi, 1991). Between the early 1980s and early 2000s, *D. v. virgifera* was accidentally introduced from North America into Europe on several occasions, leading to several independently expanding populations in Europe (Kiss *et al.*, 2005; Miller *et al.*, 2005; Ciosi *et al.*, 2008).

Since its first detection as a maize pest in the USA in 1909 (Gillette, 1912) and in Europe in 1992 (Sivcev *et al.*, 1994), numerous studies on its biology, behavioural ecology and control measures have been conducted. Indeed, nearly 900 papers have been published dealing with *D. virgifera* (search 'western corn rootworm' or '*Diabrotica virgifera*' in publication titles found in CAB Abstracts (1900–2012)). Since the 1950s, the cumulative number of bioassays exceeded 150 published papers by 2012, primarily dealing with quantifying the life history traits, with impacts of weather conditions on different life stages, the host suitability of maize hybrids as well as of potential alternative host plants, and with the development of resistances to insecticides and transgenic traits in maize (Vidal *et al.*, 2005). Standardized laboratory studies were possible due to laboratory-reared colonies of *D. v. virgifera*, such as those at the USDA-ARS North Central Agricultural Research Laboratory (NCARL; *Brookings*, South Dakota, USA). Another major advantage was the selection of a non-diapause colony of *D. v. virgifera* in the early 1970s at NCARL (Branson, 1976), which reduced generation time from many to a few months allowing four generations each year as opposed to the normal 1 year generation for wild type *D. v. virgifera*. Currently, a large proportion of laboratory studies are conducted using this non-diapause colony (Kim *et al.*, 2007; Lefko *et al.*, 2008; Meihls *et al.*, 2008). However, reliance on a long-term laboratory colony raises concerns about whether this non-diapause colony, or any other laboratory colony of *D. v. virgifera*, still reflects 'natural' *D. v. virgifera* (Kim *et al.*, 2007; Lefko *et al.*, 2008). The non-diapause colony, for example, has been in culture for more than 190 generations without out-crossings or refreshments with field-collected beetles (Kim *et al.*, 2007). Also, many of the available diapause colonies from different laboratories have been reared for more than ten generations. In contrast, the researchers that do not have access to constantly reared laboratory cultures establish short-term cultures for the duration of their usually 2–4-year-long projects, or conduct research on field-collected F_0 or on F_1 individuals.

However, most studies do not aim to compare different laboratory colonies or wild *D. v. virgifera* populations (Spencer *et al.*, 2009). Thus, the effect of the original environmental conditions, laboratory rearing or genetic differentiation between populations has seldom been addressed. Lefko *et al.* (2008) compared some fitness traits among differently reared and crossed laboratory colonies of *D. v. virgifera*. They found that particularly in early generations (e.g., during rearing from F_0 to approximately F_4 or F_5) survival rates can vary widely between colonies and between generations. Kim *et al.* (2007) investigated the genetic variability of the USDA-ARS NCARL non-diapause colony ($>F_{190}$), several diapause colonies initially collected from the USA ($>F_{22}$, F_3 to F_8), as well as some wild USA populations. Astonishingly, the neutral genetic variability revealed by microsatellite genotyping was similar among most laboratory colonies and wild populations (Kim *et al.*, 2007). A moderate loss in genetic variability (*ca.* 15–39%,) was only found in the laboratory reared ($>F_{190}$) non-diapause colony. In general, there was little evidence that the laboratory colonies underwent significant genetic bottlenecks or selection processes compared to wild populations. The results of Kim *et al.* (2007) suggest that the USA – *D. v. virgifera* colonies maintained in laboratories are usually genetically similar to each other and to wild populations, at least for the neutral genetic variability that was studied. However, establishing a relationship between

Table 1. Studied colonies of three *D. v. virgifera* populations reared at CABI, Delemont, Switzerland under identical laboratory condition; from the Central/Southeastern European population (CSE), from the Northwestern Italian population (NW I), and the Central-to-Northern US American population USA, The USA SD2 colony is a colony selected for non-diapause.

Population	Central/Southeastern European			Northwestern Italian	Central-to-Northern USA		
Tested colony	Tolna county	Timisoara county	Severnobacki county	Lombardy province	Pennsylvania state	South Dakota state	South Dakota state
Colony code	CSE1	CSE2	CSE3	NW I	USA PA	USA SD1	USA SD2
Year of field collection	2006	2006	2006	2006	2000	1986	1966
Tested generation	F_1	F_1	F_2	F_1, F_2	F_8	F_{23}	F_{191}
n pairs of adults tested	110	98	148	142, 38	146	150	196

neutral genetic variability, which might be less subject to natural selection, and phenotypic variability, which primarily results from the pressures of natural selection, remains difficult to establish. Therefore, it is largely unknown whether laboratory-reared colonies also have similar comparable phenotypes. It has been suggested that the non-diapause beetles from NCARL appear somewhat larger and less active than beetles from other colonies (Ch. Nielson, Brookings, SD, USA, 2007 pers. comm.; K. Gloyna, Sagerheide, Germany, 2008 pers. comm.). In contrast to laboratory colonies, it is well-reported that field-collected F_0 *D. v. virgifera* adults vary in their fitness (Spencer *et al.*, 2009), depending on environmental conditions or insect age at collection (Li *et al.*, 2010), nutritional value of the maize plants, and on many other factors. Whether effects of the previous field environment can still be detected as parental effects in laboratory colonies during subsequent rearing (Bernardo, 1996; Mousseau & Fox, 1998; Johnston *et al.*, 2004) is unknown for *D. v. virgifera*.

Our study used several laboratory colonies with the goal to rear, observe, and measure hypothesized differences in phenotypic traits using standardized bioassays from Li *et al.* (2009) and Toepfer *et al.* (2012). F_1 generations from the Central/Southeastern European *D. v. virgifera* population, but from different locations that present no neutral genetic differentiation (Miller *et al.*, 2006, 2007, Ciosi *et al.*, 2008) were used to investigate hypothesized parental effects originating from the field situation. An F_1 and an F_2 generation of laboratory-reared Northwest Italian *D. v. virgifera* were compared to evaluate whether researchers can reliably use F_1 generations or should rather rear *D. v. virgifera* at least up to the F_2 generation before starting bioassays. Furthermore, *D. v. virgifera* from different generations of three long-reared USA colonies were used to study hypothesized changes in phenotype over the time of rearing. Finally, colonies from genetically distinct populations (two independently founded European outbreaks and one USA population) were compared to estimate the hypothesized effect of neutral genetic differentiation between populations of origin on phenotype. This will help researchers decide whether to work with laboratory colonies or with field-collected insects in comparative studies targeting fitness, activity, or morphometric questions regarding *Diabrotica* pest species.

Materials and methods

D. v. virgifera *colonies and standard rearing conditions*

Experiments were conducted with seven laboratory-reared colonies. They originated from three genetically distinct populations (here synonym for genotypic classes, Johnston *et al.*, 2004) (table 1): the Central/Southeastern European population (CSE European), the Northwest Italian population (NW INW I) as defined by Miller *et al.* (2005), and the USA population (excluding Texas) as defined by Miller *et al.* (2006, 2009) (table 1). The neutral genetics of the CSE population were characterized by about 3.4 different alleles directly counted per locus (*DC*), and an expected heterozygosity (H_e) of about 0.45 under Hardy–Weinberg assumptions (Miller *et al.*, 2006, 2007; Ciosi *et al.*, 2008). The *DC* of the NW Italian population was about 3.3 and the H_e about 0.35 (Miller *et al.*, 2005, 2006, 2009; Ciosi *et al.*, 2008). The USA population was characterized by *DCs* from 5.3 to 9.0, allele richness *AR* from 5.3 to 8.9, H_e from 0.59 to 0.69, and the observed heterozygosity H_o from about 0.59 to 0.76 (Kim & Sappington, 2005; Miller *et al.*, 2005, 2006; Kim *et al.*, 2007; Ciosi *et al.*, 2008). Within each genetic population (CSE European, NW Italian, USA), genetic variation ranged from none to very little (Kim & Sappington, 2005; Miller *et al.*, 2005, 2006; Kim *et al.*, 2007; Ciosi *et al.*, 2008).

Three CSE European population colonies were each founded with more than 1000 adults (F_0) that were collected from heavily infested maize fields in Tolna County of southern Hungary (colony CSE1), Timisoara County of Northern Romania (colony CSE2), and Severnobacki County of Northern Serbia (colony CSE3). A single colony originated from the NW Italian population; it was established from 500 adults (F_0) collected from an infested maize field in Lombardy County (colony NW I). Three *D. v. virgifera* colonies originated from the USA; they were established from at least 1000 adults (F_0) collected from fields in Pennsylvania or South Dakota (colonies USA PA, USA SD1, and USA SD2). For dates of colony establishments refer to table 1. The number of field collect founders of each colony or population was large enough to avoid random genetic drift and inbreeding depression, according to Miyatake & Yamagishi (1999).

Before experiments, each *D. v. virgifera* colony was reared at CABI, Delemont, Switzerland under identical laboratory conditions, as per George & Ortman (1965), Branson *et al.* (1975), and Li *et al.* (2009). Eggs were incubated at a temperature of 25°C (during photophase: L) and 21°C (during scotophase: D) for 14–24 days to initiate egg hatching. About 2000–3000 untreated seeds of the maize hybrid Gavott (UFA Semences, Bussigny, Switzerland) were soaked in water for 24 h and germinated in a plastic tray (l: 330 mm, w: 190 mm, and h: 110 mm) with a gauze lid. Two to three days after germination, ready-to-hatch eggs were transferred in their overwintering soil onto a filter paper and then onto the seeds

in the plastic trays (approx. 5000 eggs per tray) and maintained at a temperature of L: D, 25°C: 21°C, and light regime of L: D, 14: 10. Emerging larvae found unlimited food (maize roots). After 14–20 days, third instar larvae were transferred along with the maize seedlings into gauze-covered cylinders (dia.: 120 mm, h: 140 mm) containing sterilized field soil for pupation (soil sieved at < 5 mm mesh size, 30% moisture). The transferred maize continued to provide food for the developing larvae until they pupated. Emerging adults were collected and either used for experiments (see below) or transferred to gauze cages (450 × 450 × 600 mm) for further mass rearing of subsequent generations according to Krysan & Miller (1986) and Singh & Moore (1999) (temperature L: D, 24–26°C: 18–20°C, 40–60% relative humidity, light regime L: D, 14: 10). For mass rearing, at least 100 mated females of each colony and rearing cycle were allowed to lay eggs into petri dishes with moist, sterilized and sieved black Chernozem field soil (< 200 µm mesh size) up to 2 months after collection. The petri dishes were changed every 2 weeks. The soil with eggs was washed through a 0.25 mm sieve with room temperature tap water. The number of recovered eggs on the sieve was estimated. Eggs were then stored in the sterile moist soil and kept at a temperature of L: D, 25: 21°C during 2 weeks for pre-diapause (Krysan, 1972; Branson, 1976). Eggs were then overwintered for diapause at 6–8°C for 5–7 months (Krysan, 1982), except for eggs from the non-diapause USA colony (USA SD2), which hatch in about 14 days, and were used for experiments or further mass rearing immediately.

Assessing phenotypic traits

Sixteen phenotypic traits were assessed according to Li *et al.* (2009, 2010): five fitness traits (fecundity, overwintering egg survival, larva-to-adult survival, egg-to-adult survival, and adult lifespan), four activity traits (proportion of adults crawling, crawling speed, proportion of adults flying, and flight take-off response), and seven morphometric traits of adults (fresh body weight, elytra length and width, pronotum length and width, head capsule width, and hind tibia length). Assessments were conducted with *D. v. virgifera* from two rearing series (= repetitions) of each colony (Begley, 2013). Data from two series were pooled per colony as traits rarely showed any difference between series within a generation. Assessments of adult *D. v. virgifera* were only conducted with young 1–6 day old individuals to reduce the influence of age and the rearing environment on shaping the phenotypic traits as investigated by Li *et al.*, (2009, 2010). Moreover, *D. v. virgifera* individuals develop and mature at different speed adding to an increasing variability in trait data with age of a colony, which renders comparisons between colonies or populations difficult (Li *et al.*, 2009, 2010). Averages, ranges, variation, and statistical tests of all trait data are presented in tables 2–5 to allow cross-checking, and the repetition of experiments by users according to Begley (2013).

Fitness traits

In the laboratory, newly emerged adults were sexed according to antenna length (Staetz *et al.*, 1976; Kuhar & Youngman, 1995) and tarsal characteristics (Hammack & French, 2007). Male–female pairs were transferred to small bioassay containers (2 daylight simulating Osram HQI-BT 400 watt lamps at light regime L: D, 14: 10; temperature L: D, 24: 18°C; relative humidity 60%). The containers consisted of two plastic urinalysis cups (dia.: 48 mm, h: 80 mm), stacked one inside the other providing approximately 175 cm^3 of space (for details see Li *et al.*, 2009; Toepfer *et al.*, 2012). The upper cup had a 10-mm hole in the bottom to give the female access to the lower, soil-filled cup for egg-laying. Abundant food was provided in each container (two soft, unripe kernels of organically-produced maize one 13 × 13 × 13 mm piece of zucchini flesh, one 13 × 13 × 13 mm piece of pumpkin flesh (Li *et al.*, 2009), and a 5 × 5 × 5 mm piece of artificial pollen diet (Branson & Jackson, 1988; Singh & Moore, 1999). A 10 × 5 × 5 mm cube of 15% water agar served as a water source for the adults. Food and agar were changed every 5–7 days. Each tested pair of *D. v. virgifera* was provided with the same amount of food, as *D. v. virgifera* fitness and activity is known to be influenced by recent diet experience (Levine *et al.*, 2002; Mabry *et al.*, 2004).

To assess adult lifespan, bioassay containers were checked daily for live and dead adults. The date of death was recorded, lifespan was calculated, and dead adults were removed and not replaced. The latter was implemented, because *D. v. virgifera* females are known to be able to lay all their eggs after one mating only (Spencer *et al.*, 2009) and because survival curves of males and females are similar (Li *et al.*, 2009, 2010). The lifespan assessment was stopped after 70 days because this period is long enough to reliably reflect the total adult lifespan (Li *et al.*, 2009). The proportion of females and males surviving until day 70 was calculated for each tested colony.

To assess fecundity, two teaspoons of moist, sterile black Chernozem field soil (sieved at 0.15 mm mesh size; 25–35 wt% moisture) were placed into the lower cup of the bioassay containers (see above) after 7 days of maturation (Branson & Johnson, 1973; Hill, 1975). Moisture remained stable due to nearly-closed environment of the double-stacked bioassay container. Then, every 14 days, the lower cup of the bioassay container (containing soil and eggs) was removed and replaced with a new one. The soil with eggs was washed with room temperature tap water through a 0.25 mm sieve, and the recovered eggs were counted. The accumulated 98-day (= total) realized fecundity was calculated per individual female for a number of colonies, but then reduced to measuring the 70-day fecundity as Li *et al.* (2009) reported a linear relationship between fecundity and age, and suggested that the 70-day fecundity is enough to reliably estimate and compare colony fecundities. Eggs were then stored in sterile moist soil and kept as described above.

To determine the overwintering survival of eggs, the soil with eggs was washed through a 0.25 mm sieve after 5 months of diapause. Egg survival was checked according to Modic *et al.* (2008) under a stereomicroscope and recorded per individual parental female of each tested colony.

To determine larva-to-adult survival, 200 successfully overwintered viable eggs were incubated at a temperature of L: D, 25: 21°C for 14–24 days to initiate egg hatching. Because not every female had laid enough eggs to obtain 200 eggs after overwintering, this portion of the study was conducted with pooled egg batches. The larvae and pupae were reared as described above. Adult emergence was recorded daily until there were four consecutive days without any emergence. The total number of emerged adults was divided by the initial number of viable eggs ($n = 200$) to calculate the hatched-larva-to-adult survival.

Table 2. Differences between F_1 colonies from different locations of the same genetic population (field influenced parental effects) assessed through average levels and variability of phenotypic traits between F_1 from Southern Hungary (colony CSE1), and from northern Romania (colony CSE2) (both Central/Southeastern European genetic population CSE). Different letters in rows indicate significant differences of mean values (small fonts) or variances (capital fonts) of traits between colonies according to fdr correction; n = number of individuals, SD = standard deviation, CV = coefficient of variation. [Note: 98 day – fecundity for CSE1: 461 ± 398 eggs with range of 0–1386 (n = 55) and CV of 0.86 versus CSE 2: 196 ± 226 eggs with range of 0–856 (n = 48) and CV of 1.15 ($P < 0.005$ between means and between variances)].

Phenotypic trait	Both sexes		Females		Males	
	F_1 (CSE1)	F_1 (CSE2)	F_1 (CSE1)	F_1 (CSE2)	F_1 (CSE1)	F_1 (CSE2)
Fitness traits						
Fecundity 70 days (eggs)						
Mean ± SD[1]			398 ± 338 b	176 ± 204 a		
Range			0–1228	0–723		
CV[2]			0.85 A	1.16 B		
n			55	48		
Egg overwintering survival (%)						
Mean ± SD[3]	25 ± 14 a	26 ± 25 a				
Range	0–60	0–82				
CV[4]	0.56 A	0.95 B				
n clutches	47	36				
Adult lifespan (days) (standardized to 70 days)						
Mean ± SD[1]	52 ± 22 b	41 ± 26 a	54 ± 22 a	47 ± 26 a	50 ± 22 b	35 ± 26 a
Range	2–70	1–70	3–70	1–70	2–70	1–70
CV[2]	0.42 A	0.65 B	0.40 A	0.55 B	0.44 A	0.75 B
n	110	98	55	49	55	49
Initial activity trait						
Proportion of adults flying (%)						
Mean ± SD[5]	93 ± 25 b	79 ± 41 a	96 ± 19 b	70 ± 46 a	90 ± 30 a	91 ± 28
CV[2]	0.27 A	0.51 B	0.20 A	0.66 B	0.33 A	0.31 A
n	104	82	53	47	51	35
Flight take – off response (s)						
Mean ± SD[1]	48 ± 73 a	58 ± 71 a	69 ± 86 a	87 ± 78 a	26 ± 46 a	21 ± 36 a
Range	1–296	1–292	1–296	1–292	1–210	1–205
CV[2]	1.51 A	1.22 A	1.25 A	0.90 A	1.79 A	1.76 A
n	103	81	53	46	50	35
Proportion of adults crawling (%)						
Mean ± SD[5]	97 ± 17 a	99 ± 11 a	96 ± 19 a	98 ± 15 a	98 ± 14 a	100 ± 0 a
CV[2]	0.17 A	0.11 A	0.20 A	0.15 A	0.14[6]	0.00[6]
n	104	82	53	47	51	35
Adult crawling speed (s/400 mm)						
Mean ± SD[1]	24 ± 18 a	31 ± 39 a	25 ± 20 a	28 ± 33 a	23 ± 16 a	33 ± 46 a
Range	9–113	12–260	10–113	13–236	9–79	12–260
CV[2]	0.75 A	1.28 A	0.79 A	1.18 A	0.70 A	1.38 B
n	101	79	51	45	50	34
Initial morphometric traits						
Adult fresh body weight (mg)						
Mean ± SD[7]	10.9 ± 1.9 a	10.3 ± 2.3 a	11.2 ± 1.7 a	10.9 ± 2.1 a	10.6 ± 2.0 a	9.8 ± 2.3 a
Range	4.2–15.3	4.9–16.4	7.6–15.3	7.0–15.3	4.2–15.1	4.9–16.4
CV[4]	0.17 A	0.22 B	0.15 A	0.19 B	0.19 A	0.23 A
n	110	98	55	49	55	49
Elytron length (mm)						
Mean ± SD[7]	4.29 ± 0.24 b	4.14 ± 0.32 a	4.38 ± 0.21 a	4.28 ± 0.27 a	4.20 ± 0.24 b	4.00 ± 0.29 a
Range	3.61–4.77	3.37–4.90	3.79–4.77	3.86–4.90	3.61–4.65	3.37–4.59
CV[4]	0.06 A	0.08 B	0.05 A	0.06 A	0.06 A	0.07 A
n	110	98	55	49	55	49
Elytra width (mm)						
Mean ± SD[8]	2.50 ± 0.12 a	2.43 ± 0.15 a	2.52 ± 0.12 a	2.48 ± 0.14 a	2.47 ± 0.13 a	2.38 ± 0.15 a
Range	2.08–2.75	1.96–2.82	2.20–2.75	2.20–2.82	2.08–2.75	1.96–2.63
CV[4]	0.05 A	0.06 A	0.05 A	0.06 A	0.05 A	0.06 A
n	110	98	55	49	55	49
Pronotum length (mm)						
Mean ± SD[7]	1.23 ± 0.07 a	1.19 ± 0.08 a	1.24 ± 0.06 a	1.21 ± 0.07 a	1.22 ± 0.07 a	1.18 ± 0.09 a
Range	1.04–1.41	1.04–1.41	1.10–1.35	1.04–1.35	1.04–1.41	1.04–1.41
CV[4]	0.05 A	0.07 B	0.05 A	0.06 A	0.06 A	0.07 A
n	110	98	55	49	55	49

Table 2. (*Cont.*)

Phenotypic trait	Both sexes		Females		Males	
	F_1 (CSE1)	F_1 (CSE2)	F_1 (CSE1)	F_1 (CSE2)	F_1 (CSE1)	F_1 (CSE2)
Pronotum width (mm)						
Mean ± SD[7]	1.51 ± 0.08 a	1.47 ± 0.11 a	1.54 ± 0.07 a	1.51 ± 0.09 a	1.48 ± 0.08 a	1.43 ± 0.12 a
Range	1.29–1.65	1.22–1.71	1.35–1.65	1.35–1.71	1.29–1.65	1.22–1.65
CV[4]	0.05 A	0.08 B	0.05 A	0.06 B	0.05 A	0.08 B
n	110	98	55	49	55	49
Head capsule width (mm)						
Mean ± SD[8]	1.20 ± 0.06 b	1.16 ± 0.07 a	1.20 ± 0.06 a	1.18 ± 0.07 a	1.19 ± 0.06 b	1.15 ± 0.08 a
Range	1.10–1.35	0.98–1.35	1.10–1.35	1.04–1.35	1.10–1.29	0.98–1.29
CV[4]	0.05 A	0.06 A	0.05 A	0.06 A	0.05 A	0.07 A
n	110	98	55	49	55	49
Hind tibia length (mm)						
Mean ± SD[7]	1.80 ± 0.10 b	1.74 ± 0.11 a	1.81 ± 0.11 b	1.76 ± 0.12 a	1.79 ± 0.09 b	1.72 ± 0.11
Range	1.50–2.00	1.30–2.00	1.50–2.00	1.50–2.00	1.60–2.00	1.30–1.90
CV[4]	0.05 A	0.07 B	0.06 A	0.07 A	0.05 A	0.06 A
n	110	98	55	49	55	49
Overall phenotypic *CV*						
Mean ± SD[6]	0.38 ± 0.46 A	0.49 ± 0.52 B	0.36 ± 0.41 A	0.44 ± 0.47 B	0.34 ± 0.55 A	0.46 ± 0.63 B

[1] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM for non-normally distributed data. Distribution considered as Poisson log-linear link function.
[2] Difference in variation through comparing variances using Levine's tests in case of non-normally distributed data.
[3] No comparison because of only few data values for each colony due to pooled rearing of larvae.
[4] Difference in variation through comparing variances using F – statistics in case of normally distributed data (with or without transformation).
[5] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM because of non-normally distributed data. Distribution considered as binary probit link function.
[6] Differences of overall *CV* according to non-parametric related samples sign test
[7] fdr-corrected multiple Games–Howell pairwise comparison *post hoc* test after ANCOVA because of close-to-normally distributed data (with or without transformation) and unequal homogeneity of variances.
[8] fdr-corrected multiple comparison Tukey *post hoc* range test after ANCOVA because of close-to-normally distributed data (with or without transformation) and equal homogeneity of variances.

Egg-to-adult survival was calculated by combining the data from the overwintering survival of eggs with the survival from hatched larvae via pupae until adulthood.

Initial activity traits

Crawling activity of young adults was assessed as a measure of short distance within-field dispersal; and flight activity as a measure of dispersal capability in general. Crawling speed and the proportion of adults crawling were measured using the methodology of Mabry *et al.* (2004) and Li *et al.* (2010). Flight take-off response and the proportion of adults flying were measured as by Duan *et al.*, (1998); Toepfer *et al.*, (2005); and Li *et al.*, (2010). Measurements were made using young adults (6 day old = initial activity trait), because activity measures on young adults better reflect activity differences between individuals or colonies than measures on mature adults which are much influenced by nutritional status and egg load (Li *et al.*, 2010). The trials were conducted under laboratory conditions of 24°C, 50–60% relative humidity, and simulated daylight (2 Osram HQI-BT 400 W daylight lamps). They were always conducted between 14:00 and 16:00 as both temperature (VanWoerkom *et al.*, 1980), and time of the day (Coats *et al.*, 1986) can influence activity.

As for crawling, a 400-mm long crawling section was marked on a 700-mm long transparent plastic tube of 9 mm diameter. The plastic tube was vertically fixed to a ring stand. A plastic vial containing an adult was slipped under the open bottom end of the tube allowing the adult to crawl into the tube and upwards. Once the adult reached the 400 mm section, the time from the starting to the end point was recorded as well as the incidence of crawling, not crawling or crawling but not finishing the 400 mm distance within 300 s. The trial ended when the adult had crawled the 400 mm or when 300 s had elapsed (according to Li *et al.*, 2010). Tested adults were returned to the rearing containers. The proportion of adults crawling, and their mean crawling speed were calculated for young adult males and females by colony. As crawling and flight activity appeared highly correlated (Li *et al.*, 2010), the crawling trait measure was skipped in later colony assessments.

To assess flight activity, flight stands were used that consisted of a wooden pin (h: 40 mm, dia.: 10 mm) fixed onto the end of an inverted white plastic funnel (h: 160 mm, dia.: 135 mm at base). The base of the funnel was surrounded with water to prevent the adults from walking off of the stand. An individual was gently released onto the base of the funnel using an aspirator. Following release, the incidence of take-off and the time between release and take-off were recorded. A trial ended when the adults flew off the stand or when 300 s had elapsed without take-off (Toepfer *et al.*, 2005; Li *et al.*, 2010). Tested insects were returned to their bioassay containers. The proportion of adults flying and the mean time

Table 3. Differences between the F_1 and F_2 generation of the same genetic population (effects of early generation laboratory rearing), assessed through average levels and variability of phenotypic traits between a laboratory-reared F_1 and F_2 of *D. v. virgifera* originating from a field-collected F_0 of the Northwestern Italian genetic population (NW I). Different letters in rows indicate significant differences of mean values (small fonts) or variances (CAPITAL FONTS) of traits between colonies according to fdr correction; *n* = number of individuals, SD = standard deviation, *CV* = coefficient of variation.

Phenotypic trait	Both sexes		Females		Males	
	F_1 (NW I)	F_2 (NW I)	F_1 (NW I)	F_2 (NW I)	F_1 (NW I)	F_2 (NW I)
Fitness traits						
Fecundity 70 days (eggs)						
Mean ± SD[1]			521 ± 338 a	747 ± 442 b		
Range			0–1327	0–1323		
CV[2]			0.65 A	0.59 A		
n			71	19		
Egg overwintering survival (%)						
Mean ± SD[3]	24 ± 15 a	65 ± 11 b				
Range	0–60	51–90				
CV[4]	0.60 B	0.17 A				
n clutches	64	16				
Hatched-larva-to-adult survival (%)						
Mean ± SD[5]	34 ± 4	10 ± 0				
Range	3–39	10–10				
CV[6]	0.13	0.00				
n clutches	64	16				
Egg-to-adult survival (%)						
Mean ± SD[3]	8 ± 5 a	7 ± 1 a				
Range	0–19	5–9				
CV[6]	0.59	0.17				
n clutches	64	16				
Adult lifespan (days) (standardized to 70 days)						
Mean ± SD[1]	59 ± 19 a	62 ± 18 a	57 ± 19 a	68 ± 5 b	60 ± 19 a	57 ± 24 a
Range	3–70	3–70	7–70	55–70	3–70	3–70
CV[2]	0.33 A	0.29 A	0.34 B	0.07 A	0.32 A	0.43 A
n	142	38	71	19	71	19
Initial activity trait						
Proportion of adults flying (%)						
Mean ± SD[7]	99 ± 8 a	94 ± 23 a	99 ± 12 a	89 ± 32 a	100 ± 0 a	100 ± 0 a
CV[2]	0.09 A	0.25 B	0.12 A	0.35 B	0.0 A	0.0 A
n	140	36	70	19	70	17
Flight take-off response (s)						
Mean ± SD[1]	20 ± 36 a	17 ± 26 a	29 ± 43 a	28 ± 31 a	12 ± 25 a	5 ± 7 a
Range	1–247	1–112	1–247	2–112	1–176	1–28
CV[2]	1.78 A	1.50 A	1.50 A	1.11 A	2.11 A	1.40 A
n	140	36	70	19	70	17
Initial morphometric traits						
Adult fresh body weight (mg)						
Mean ± SD[3]	10.3 ± 1.5 b	9.0 ± 2.0 a	10.8 ± 1.5 b	9.9 ± 2.0 a	9.7 ± 1.3 b	8.2 ± 1.6 a
Range	6.2–13.6	4.5–13.0	8.1–13.6	6.2–13.0	6.2–12.6	4.5–10.7
CV[4]	0.15 A	0.22 B	0.14 A	0.20 B	0.14 A	0.19 A
n	142	38	71	19	71	19
Elytron length (mm)						
Mean ± SD[8]	4.15 ± 0.29 a	4.21 ± 0.29 b	4.26 ± 0.26 a	4.32 ± 0.32 a	4.04 ± 0.28 a	4.11 ± 0.22 b
Range	2.45–4.77	3.67–4.84	3.49–4.77	3.79–4.84	2.45–4.47	3.67–4.41
CV[4]	0.07 A	0.07 A	0.06 A	0.08 B	0.07 A	0.05 A
n	142	38	71	19	71	19
Elytra width (mm)						
Mean ± SD[8]	2.38 ± 0.16 a	2.42 ± 0.17 b	2.44 ± 0.14 a	2.46 ± 0.20 a	2.32 ± 0.14 a	2.38 ± 0.14 b
Range	1.90–2.69	1.96–2.75	1.96–2.69	2.08–2.75	1.90–2.63	1.96–2.63
CV[4]	0.07 A	0.07 A	0.06 A	0.08 B	0.06 A	0.06 A
n	142	38	71	19	71	19
Overall phenotypic *CV*						
Mean ± SD[9]	0.52 ± 0.60 A	0.42 ± 0.51 A	0.48 ± 0.51 A	0.33 ± 0.39 A	0.54 ± 0.89 A	0.43 ± 0.56 A

[1] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM for non-normally distributed data. Distribution considered as Poisson log-linear link function.
[2] Difference in variation through comparing variances using Levine's tests in case of non-normally distributed data.
[3] fdr-corrected multiple Games–Howell pairwise comparison *post hoc* test after ANCOVA because of close-to-normally distributed data (with or without transformation) and unequal homogeneity of variances.
[4] Difference in variation through comparing variances using F-statistics in case of normally distributed data (with or without transformation).
[5] No comparison because of only few data values for each colony due to pooled rearing of larvae.
[6] Differences in variances not tested because of lack of enough spread/level pairs.
[7] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM because of non-normally distributed data. Distribution considered as binary probit link function.
[8] fdr-corrected multiple comparison Tukey *post hoc* range test after ANCOVA because of close-to-normally distributed data (with or without transformation) and equal homogeneity of variances.
[9] Differences of overall *CV* according to non-parametric related samples sign test.

Table 4. Differences between different generations of the same population (effects of long-term laboratory rearing) assessed through average levels and variability of phenotypic traits between long-term reared *D. v. virgifera* colonies of the USA genetic population, i.e., an F_8 from Pennsylvania, F_{23} and F_{191} from two fields of Moody County in South Dakota. F_{191} had been selected for non-diapause from an originally diapausing strain. Different letters in rows indicate significant differences of mean values (small fonts) or variances (capital fonts) of traits between colonies according to fdr correction; *n* = number of individuals, SD = standard deviation, *CV* = coefficient of variation. [Note: 98 day – fecundity for F_{23} USA: 537 ± 390 eggs with range of 0–1438 (n = 73) and CV of 0.73 versus F_{191} USA: 703 ± 488 eggs with range of 0–1814 (n = 75) and CV of 0.69 ($P < 0.005$ between means and $P > 0.005$ between variances)].

Phenotypic trait	Both sexes			Females			Males		
	F_8 (USA PA)	F_{23} (USA SD1)	F_{191} (USA SD2)	F_8 (USA PA)	F_{23} (USA SD1)	F_{191} (USA SD2)	F_8 (USA PA)	F_{23} (USA SD1)	F_{191} (USA SD2)
Fitness traits									
Fecundity 70 days (eggs)									
Mean ± SD[1]				691 ± 396 b	481 ± 353 a	613 ± 408 ab			
Range				0–1489	0–1438	0–1403			
CV[2]				0.57 A	0.73 A	0.67 A			
n				73	73	75			
Egg overwintering survival (%)									
Mean ± SD[3]	43 ± 27 b	18 ± 13 a	24 ± 17 ab						
Range	0–100	0–56	0–78						
CV[4]	0.63 A	0.71 B	0.71 B						
n clutches	71	62	69						
Adult lifespan (days) (standardized to 70 days)									
Mean ± SD[1]	59 ± 18 b	51 ± 25 a	56 ± 21 ab	58 ± 16 a	52 ± 23 a	57 ± 20 a	60 ± 19 b	51 ± 26 a	56 ± 21 ab
Range	3–70	1–70	2–70	3–70	1–70	3–70	3–70	2–70	2–70
CV[2]	0.30 A	0.49 B	0.37 AB	0.28 A	0.45 B	0.35 AB	0.32 A	0.52 B	0.39 AB
n	146	150	196	73	75	99	73	75	98
Initial activity trait									
Proportion of adults flying (%)									
Mean ± SD[5]	82 ± 39 c	67 ± 47 b	48 ± 50 a	71 ± 46 c	51 ± 50 b	29 ± 46 a	93 ± 26 b	83 ± 38 ab	67 ± 47 a
CV[2]	0.48 A	0.71 B	1.05 B	0.65 A	0.98 B	1.58 B	0.28 A	0.45 B	0.70 C
n	141	138	189	72	72	97	69	66	92
Flight take – off response (seconds)									
Mean ± SD[1]	49 ± 79 a	65 ± 80 ab	75 ± 91 b	51 ± 77 a	83 ± 90 a	56 ± 88 a	46 ± 82 a	47 ± 63 a	94 ± 91 b
Range	1–299	1–288	1–300	1–297	1–288	1–297	1–299	1–251	1–300
CV[2]	1.63 B	1.22 A	1.22 A	1.50 A	1.08 A	1.58 A	1.79 A	1.33 A	0.96 A
n	139	133	182	71	67	92	68	66	90
Proportion of adults crawling (%)									
Mean ± SD[5]		99 ± 12 a	100 ± 0 a		99 ± 12 a	100 ± 0 a		98 ± 12 a	100 ± 0 a
CV[6]		0.12	0.0		0.12	0.0		0.12	0.0
n		138	189		72	97		66	92
Adult crawling speed (seconds/400 mm)									
Mean ± SD[1]		31 ± 31 a	35 ± 32 a		38 ± 40 a	34 ± 23 a		24 ± 11 a	36 ± 40 b
Range		12–271	12–280		13–271	14–163		12–58	12–280
CV[2]		0.98 A	0.93 A		1.05 B	0.69 A		0.46 A	1.11 B
n		134	148		70	94		64	90
Initial morphometric traits									
Adult fresh body weight (mg)									
Mean ± SD[7]	10.9 ± 2.0 b	9.4 ± 1.8 a	11.7 ± 2.2 c	11.6 ± 2.0 b	9.9 ± 1.9 a	12.0 ± 2.1 b	10.1 ± 1.8 b	8.8 ± 1.6 a	11.3 ± 2.3 c
Range	6.3–20.0	3.4–18.8	6.4–18.0	8.0–20.0	6.3–18.8	7.3–18.0	6.3–15.5	3.4–12.5	6.4–18.0
CV[4]	0.19 A	0.20 A	0.19 A	0.17 A	0.19 A	0.17 A	0.18 A	0.18 A	0.20 A
n	146	149	196	73	75	98	73	74	98

Elytron length (mm)									
Mean ± SD[3]	4.19 ± 0.24 b	4.06 ± 0.29 a	4.32 ± 0.28 c	4.32 ± 0.20 a	4.16 ± 0.28 a	4.39 ± 0.28 a	4.05 ± 0.20 b	3.95 ± 0.25 a	4.25 ± 0.27 c
Range	3.55–4.65	3.12–4.65	3.43–5.08	3.86–4.65	3.43–4.65	3.73–5.08	3.55–4.53	3.12–4.41	3.43–4.77
CV[4]	0.06 A	0.07 A	0.07 A	0.05 A	0.07 B	0.06 AB	0.05 A	0.06 A	0.06 A
n	146	150	196	73	75	98	73	75	98
Elytra width (mm)									
Mean ± SD[7]	2.37 ± 0.15 a	2.38 ± 0.14 a	2.53 ± 0.16 b	2.44 ± 0.14 a	2.42 ± 0.15 a	2.56 ± 0.15 b	2.30 ± 0.12 a	2.35 ± 0.13 a	2.49 ± 0.16 b
Range	1.96–2.75	2.02–2.75	1.96–2.94	2.20–2.75	2.02–2.75	2.02–2.94	1.96–2.51	2.02–2.63	1.96–2.88
CV[4]	0.06 A	0.06 A	0.06 A	0.06 A	0.06 A	0.06 A	0.05 A	0.06 A	0.06 A
n	146	150	196	73	75	98	73	75	98
Pronotum length (mm)									
Mean ± SD[7]		1.15 ± 0.07 a	1.21 ± 0.07 a		1.17 ± 0.06 a	1.22 ± 0.06 a		1.13 ± 0.07 a	1.20 ± 0.08 a
Range		0.98–1.29	0.98–1.35		0.98–1.29	1.04–1.35		0.98–1.22	0.98–1.35
CV[4]		0.06 A	0.06 A		0.05 A	0.05 A		0.06 A	0.06 A
n		150	196		75	98		75	98
Pronotum width (mm)									
Mean ± SD[7]		1.43 ± 0.09 a	1.51 ± 0.10 b		1.47 ± 0.08 a	1.54 ± 0.10 b		1.38 ± 0.08 a	1.49 ± 0.09 b
Range		1.16–1.65	1.22–1.71		1.22–1.65	1.29–1.71		1.16–1.59	1.22–1.65
CV[4]		0.07 A	0.06 A		0.06 A	0.06 A		0.06 A	0.06 A
n		150	196		75	98		75	98
Head capsule width (mm)									
Mean ± SD[7]		1.15 ± 0.06 a	1.24 ± 0.07 b		1.17 ± 0.06 a	1.24 ± 0.07 b		1.14 ± 0.06 a	1.24 ± 0.06 b
Range		0.98–1.29	1.04–1.41		0.98–1.29	1.04–1.41		1.04–1.29	1.10–1.35
CV[4]		0.05 A	0.05 A		0.05 A	0.06 A		0.06 A	0.05 A
n		150	196		75	98		75	98
Hind tibia length (mm)									
Mean ± SD[7]		1.73 ± 0.11 a	1.81 ± 0.12 b		1.74 ± 0.10 a	1.82 ± 0.12 b		1.72 ± 0.11 a	1.81 ± 0.12 b
Range		1.30–1.90	1.40–2.10		1.40–1.90	1.40–2.00		1.30–1.90	1.50–2.10
CV[4]		0.06 A	0.07 A		0.06 A	0.07 A		0.06 A	0.07 A
n		150	196		75	98		75	98
Overall phenotypic *CV*									
Mean ± SD[8]	0.49 ± 0.55 A	0.39 ± 0.42 A	0.37 ± 0.41 A	0.46 ± 0.51[9]	0.38 ± 0.41[9]	0.38 ± 0.47[9]	0.48 ± 0.74 A	0.28 ± 0.41 A	0.30 ± 0.40 A

[1] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM for non-normally distributed data. Distribution considered as Poisson loglinear link function.
[2] Difference in variation through comparing variances using Levine's tests in case of non-normally distributed data.
[3] fdr-corrected multiple Games–Howell pairwise comparison *post hoc* test after ANCOVA because of close-to-normally distributed data (with or without transformation) and unequal homogeneity of variances.
[4] Difference in variation through comparing variances using F – statistics in case of normally distributed data (with or without transformation).
[5] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM because of non-normally distributed data. Distribution considered as binary probit link function.
[6] Differences in variances not tested because of lack of enough spread/level pairs.
[7] fdr-corrected multiple comparison Tukey *post hoc* range test after ANCOVA because of close-to-normally distributed data (with or without transformation) and equal homogeneity of variances.
[8] Differences of overall *CV* according to non-parametric related samples sign test.
[9] Differences according to related samples Friedman's two-way ANOVA by ranks. Here significant effect $P = 0.018$.

Table 5. Differences between colonies from genetically different populations assessed through average levels and variability of phenotypic traits between *D. v. virgifera* from the Central/Southeastern European genetic population (CSE, F_2), the Northwestern Italian genetic population (NW I, F_2), and the USA genetic population (USA, F_8). Different letters in rows indicate significant differences of mean values (small fonts) or variances (CAPITAL FONTS) of traits between colonies according to fdr correction; *n* = number of individuals, SD = standard deviation, *CV* = coefficient of variation.

Phenotypic trait	Both Sexes			Females			Males		
	NW I F_2	CSE F_2	USA F_8	NW I F_2	CSE F_2	USA F_8	NW I F_2	CSE F_2	USA F_8
Fitness traits									
Fecundity 70 days (eggs)									
Mean ± SD[1]				747 ± 442 a	579 ± 361 a	691 ± 396 a			
Range				0–1323	0–1507	0–1489			
CV[2]				0.59 A	0.62 A	0.57 A			
n				19	74	73			
Egg overwintering survival (%)									
Mean ± SD[3]	65 ± 11 c	42 ± 17 a	43 ± 27 b						
Range	51–90	4–82	0–100						
CV[2]	0.17 A	0.41 AB	0.63 B						
n clutches	16	70	71						
Hatched-larva-to-adult survival (%)									
Mean ± SD[4]	10 ± 0	18 ± 12	21 ± 3						
Range	10–10	6–29	19–24						
CV[5]	0.00	0.67	0.13						
n clutches	16	70	72						
Egg-to-adult survival (%)									
Mean ± SD[3]	7 ± 1 a	7 ± 6 a	9 ± 5 a						
Range	5–9	0–19	0–19						
CV[5]	0.17	0.79	0.56						
n clutches	16	70	71						
Adult lifespan (days) (standardized to 70 days)									
Mean ± SD[1]	62 ± 18 a	61 ± 18 a	59 ± 18 a	68 ± 5 a	59 ± 18 a	58 ± 16 a	57 ± 24 a	62 ± 18 a	60 ± 19 a
Range	3–70	4–70	3–70	55–70	4–70	3–70	3–70	4–70	3–70
CV[2]	0.29 A	0.29 A	0.30 A	0.07 A	0.30 B	0.28 B	0.43 B	0.28 A	0.32 A
n	38	148	146	19	74	73	19	74	73
Initial activity trait									
Proportion of adults flying (%)									
Mean ± SD[6]	94 ± 23 a	90 ± 31 a	82 ± 39 a	89 ± 32 a	85 ± 36 a	71 ± 46 a	100 ± 0 a	94 ± 23 a	93 ± 26 a
CV[2]	0.25 A	0.34 AB	0.48 B	0.35 A	0.42 AB	0.65 B	0.0 A	0.24 B	0.28 B
n	36	145	146	19	73	72	17	72	69
Flight take – off response (seconds)									
Mean ± SD[1]	17 ± 26 a	41 ± 61 b	49 ± 79 b	28 ± 31 a	51 ± 62 a	51 ± 77 a	5 ± 7 a	30 ± 59 a	46 ± 82 a
Range	1–112	1–290	1–299	2–112	1–263	1–297	1–28	1–290	1–299
CV[2]	1.50 A	1.49 B	1.63 C	1.11 A	1.20 B	1.50 C	1.40 A	1.96 B	1.79 B
n	36	144	139	19	73	71	17	71	68
Initial morphometric traits									
Adult fresh body weight (mg)									
Mean ± SD[7]	9.0 ± 2.0 a	11.7 ± 1.9 c	10.9 ± 2.0 b	9.9 ± 2.0 a	12.3 ± 1.6 b	11.6 ± 2.0 a	8.2 ± 1.6 a	11.0 ± 1.9 c	10.1 ± 1.8 b
Range	4.5–13.0	4.9–18.2	6.3–20.0	6.2–13.0	9.5–18.2	8.0–20.0	4.5–10.7	4.9–15.6	6.3–15.5
CV[8]	0.22 A	0.16 A	0.19 A	0.20 A	0.13 A	0.17 A	0.19 A	0.17 A	0.18 A
n	38	148	146	19	74	73	19	74	73
Elytron length (mm)									
Mean ± SD[8]	4.21 ± 0.29 a	4.36 ± 0.21 b	4.19 ± 0.24 a	4.32 ± 0.32 a	4.45 ± 0.15 a	4.32 ± 0.20 a	4.11 ± 0.22 a	4.26 ± 0.22 b	4.05 ± 0.20 a
Range	3.67–4.84	3.67–4.90	3.55–4.65	3.79–4.84	4.10–4.90	3.86–4.65	3.67–4.41	3.67–4.71	3.55–4.53
CV[8]	0.07 B	0.05 A	0.06 AB	0.08 B	0.03 A	0.05 AB	0.05 A	0.05 A	0.05 A
n	38	148	146	19	74	73	19	74	73

Elytra width (mm)									
Mean ± SD[3]	2.42 ± 0.17 ab	2.47 ± 0.12 b	2.37 ± 0.15 a	2.46 ± 0.20 ab	2.51 ± 0.10 b	2.44 ± 0.14 a	2.38 ± 0.14 b	2.42 ± 0.12 b	2.30 ± 0.12 a
Range	1.96–2.75	2.08–2.82	1.96–2.75	2.08–2.75	2.26–2.82	2.20–2.75	1.96–2.63	2.08–2.69	1.96–2.51
CV[8]	0.07 B	0.05 A	0.06 AB	0.08 B	0.04 A	0.06 A	0.06 A	0.05 A	0.05 A
n	38	148	146	19	74	73	19	74	73
Overall phenotypic *CV*									
Mean ± SD[9,10]	0.42 ± 0.51 A	0.44 ± 0. 51 A	0.49 ± 0.55 A	0.33 ± 0.39 A	0.39 ± 0.41 A	0.46 ± 0.51 A	0.43 ± 0.56 A	0.50 ± 0.82 A	0.48 ± 0.74 A

[1] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM for non-normally distributed data. Distribution considered as Poisson log-linear link function.
[2] Difference in variation through comparing variances using Levine's tests in case of non-normally distributed data.
[3] fdr-corrected multiple Games–Howell pairwise comparison *post hoc* test after ANCOVA because of close-to-normally distributed data (with or without transformation) and unequal homogeneity of variances.
[4] No comparison because of only few data values for each colony due to pooled rearing of larvae.
[5] Differences in variances not tested because of lack of enough spread/level pairs.
[6] fdr-corrected multiple pairwise contrast comparisons with sequential Sidak procedure after GZLM because of non-normally distributed data. Distribution considered as binary probit link function.
[7] fdr-corrected multiple comparison Tukey *post hoc* range test after ANCOVA because of close-to-normally distributed data (with or without transformation) and equal homogeneity of variances.
[8] Difference in variation through comparing variances using F – statistics in case of normally distributed data (with or without transformation).
[9] Differences of overall *CV* according to non-parametric related samples sign test.
[10] Differences according to related samples Friedman's two-way ANOVA by ranks.

until take-off (for those that flew) were calculated for young adult males and females by colony.

Initial morphometric traits

In total, seven commonly used morphometric traits were measured on each individual *D. v. virgifera* within 24 h following adult emergence (= initial morphometric traits without feeding), because measures on young adults more easily allow the detection of differences between individuals or colonies than measures on mature adults which are more variable (Li *et al.*, 2009, 2010). The tested young adults were assumed to no longer be teneral, as they were fully coloured and did not have the light grey and soft body typical of newly emerged adults.

Fresh body weight was measured by transferring adults into a small plastic container (h: 50 mm, dia.: 27 mm) and weighing them on a 0.1 mg–160 g precision scale (Fox & Czesak, 2006) and then returning them to the rearing container.

Other measured traits included: head capsule width including eyes (Branson & Ortman, 1970; Branson & Sutter, 1985), pronotum width and length, elytra width (i.e., single measurement of both elytra together across the dorsum), elytron length (randomly either left or right, Mabry *et al.*, 2004), as well as hind tibia length (randomly either left or right, Jenner & Kuhlmann, 2006). Adults were placed on a cool pad (cool but not frozen Icepack, Migros, Delemont, Switzerland) to limit their activity during the measurements with a micrometer scale to the nearest 0.06 mm under a stereomicroscope (16× magnification) (Li *et al.*, 2010). The individuals were returned to the bioassay containers.

Comparing averages of phenotypic traits

Averages of the fitness, activity, and morphometric trait levels were calculated for each colony and, per female, per male and for pooled male and female data. Distributions of these data were investigated using histograms as well as normal and detrended normal probability $Q - Q$ plots (Kinnear & Gray, 2000).

Data on fresh body weight, elytra length and width, head capsule width, pronotum length and width, and hind tibia length had normal distributions. Data on elytron length and elytra width were standardized ((*Trait data – Mean of trait data*)/*Variance of trait data*).

Data on overwintering survival of eggs as well as egg-to-adult survival were normally distributed after $\mathrm{sqrt}(x+1)$ transformation.

Data on adult lifespan, fecundity, flight take-off response, and crawling speed were Poisson distributed. Proportion of adults flying and crawling followed a binomial distribution. Larval-to-adult survival had too few data points to check for normality, and to allow parametric tests.

The influence of the independent explanatory factors 'colony origin', 'genetic population', 'generation', 'sex' and their interactions were tested on each trait (dependent factor). For normally distributed trait data, ANCOVA (Univariate general linear modelling) was applied. Since trait correlations can seriously confound factorial analyses of individual traits (Lande & Arnold, 1983), associations between phenotypic traits, i.e., the bivariate correlations published by Li *et al.* (2009, 2010), were taken as covariates in the analyses (i.e., the fresh body weight with pronotum length, elytron length and elytra width, and vice versa; pronotum length with pronotum width

and vice versa; the egg overwintering survival with fecundity).

For non-normally distributed trait data, generalized linear models (GZLM) were applied taking the distribution type of trait data into account (e.g., adult lifespan: Poisson distribution with a log linear link function; fecundity: Poisson, log linear; proportion of adults flying and crawling: binary, probit; flight take-off and crawling speed: both Poisson, log linear). During this procedure, correlated traits were considered as covariates (e.g., adult lifespan with fecundity; flight take-off response with pronotum length; crawling speed with body weight, hind tibia length, and pronotum width).

Once a significant factorial effect was detected by the above described models, the averages of the phenotypic trait levels were compared between colonies. For this, Tukey *post hoc* range test was used in the case of normally distributed trait data and equal homogeneity of variances (Kinnear & Gray, 2000); the Games–Howell pairwise comparison *post hoc* test in the case of normally distributed trait data with unequal homogeneity of variances (Games & Howell, 1976); or multiple pairwise contrast comparisons with a sequential Sidak procedure (in GZLM) in the case of non-normally distributed trait data. Due to the large number of compared factors, the *P*-values obtained from the *posthoc* tests were adjusted to *q*-values to reduce the probability of Type I errors of *P* values using the Benjamini–Hochberg method (Benjamini & Hochberg, 1995) through the package *Q*-VALUE (Storey, 2002; Storey & Tibshirani, 2003) in R (R Development Core Team, 2009).

Comparing variability in phenotypic traits

Standard deviations and variances of data were computed for the fitness, activity, and morphometric traits for each colony and per female, per male and for pooled male and female data (Valladares *et al.*, 2006). Distributions of trait data were investigated using histograms as well as normal and detrended normal probability $Q - Q$ plots (Kinnear & Gray, 2000).

Variability of each trait was compared with F – statistics for homogeneity of variances (based on means) in the case of normally distributed data, and Levine's tests for homogeneity of variances in the case of non-normally distributed data. Due to the large number of compared traits, a false discovery rate (fdr) analyses was applied to reduce the number of false *P* values <0.05 from the variance tests (see above).

To understand the standard deviation in the context of a mean of data, the coefficient of variation (*CV*) was calculated as follows: *CV* = *Standard deviation of trait*/*Mean of trait* (Krebs, 1994). As the *CV* is a dimensionless number (independent from units or widely different mean values), the *CV* of tested traits could be averaged per colony to characterize the overall phenotypic variability. This overall phenotypic variability was compared between colonies using related samples Wilcoxon Signed Rank test.

Results

Phenotypic traits

Fitness traits

D. v. virgifera females laid 565 ± 174 (mean ± SD) eggs on average across all colonies under standardized laboratory conditions within 70 days of age (tables 2–5). At 70 days, the maximum individual fecundity was 1507 eggs (found in a CSE 3 F_2 colony). At 98 days, the maximum was 1814 eggs (found in a USA F_{191} colony) which may be considered as the approximate maximum potential fecundity of this species. A small proportion of females in each colony did not lay any eggs.

Across all colonies, 38 ± 17% of eggs successfully overwintered under standardized laboratory conditions, the range for clutches was from 0 to 100%. Larva-to-adult survival was 19 ± 9% with a maximum of 39% and a minimum of 6%. Egg-to-adult survival was 7 ± 1% with a maximum of 19% and a minimum of 0%. Since adult lifespan was standardized to 70 days, no data on maximum lifespan were obtained. Average standardized lifespan was 56 ± 7 days across colonies (females 57.6 ± 6.5 days, males 54.8 ± 8.0 days).

The average generational growth rate (i.e., the net reproductive rate) was 27 ± 2 times across colonies under standardized laboratory conditions, with a maximum rate of 100 for a few *D. v. virgifera* pairs (found in a CSE F_2 colony and in a USA F_8 colony) and a minimum rate of 0 for a few other pairs.

Initial activity traits

On average across colonies, 99 ± 1%, of the tested young (i.e., 7-day-old) adults crawled, and on average escaped the 400-mm long tube within 30 ± 5 s (speed = 13 mm s^{-1} or 8 m min^{-1}). Among females, 98 ± 2% crawled and escaped the tube within 32 ± 6 s. Among males, 99 ± 1% crawled and escaped the tube this within 29 ± 6 s. The fastest adults needed only about 9–10 s to travel the 400 mm tube distance (speed = 40–44 mm s^{-1} or about 25 m min^{-1}). Such individuals were found among both sexes and in each colony.

On average across colonies, 83 ± 17% of the young adults flew off the flight stands under standardized laboratory conditions. The mean time between release and take-off was 44 ± 24 s. Among females, 75 ± 22% took off, on average this occurred 54 ± 21 s after they were released on the stand. Males flew off more frequently than females (91 ± 10%, t-test = 9, df $_{1;997}$, $P = 0.003$) and faster (within on average 33 ± 27 s, $t = -10.5$, df $_{1;890}$, $P = 0.004$). The very fastest adults needed only *ca.* 1 s to take off, and such individuals were found among both sexes and in each colony.

Initial morphometric traits

Young, i.e., 1-day-old adults weighed 10.4 ± 1.1 mg on average across colonies under standardized laboratory conditions. Young females were heavier (11.01 ± 0.9 mg) than young males (9.8 ± 1.1 mg, t-test, $P = 0.007$). The heaviest young female was 20 mg (found in a USA F_8 colony) and the heaviest young male was weighed 18 mg (found in a USA F_{191} colony). The lightest young female was 6.2 mg (found in a NW I F_2 colony), the lightest young male was 3.4 mg (found in a USA F_{23} colony).

One-day-old adults had 4.2 ± 1.1 mm long and 2.43 ± 0.13 mm wide elytra, a 1.2 ± 0.03 mm long and 1.48 ± 0.05 mm wide pronotum, a 1.19 ± 0.03 mm wide head capsule, and 1.77 ± 0.04 mm long hind tibias. For separate average measures by sexes refer to tables 2–5. The longest female elytron was 5.1 mm, and the longest male elytron was 4.77 mm (both from the USA F_{191} colony). The widest female elytra were 2.94 mm and the widest male elytra were 2.88 mm (both from the USA F_{191} colony). The shortest female elytron was 3.43 mm

(from the USA F_{23} colony), and the shortest male elytron was 2.45 mm (from the NW I F_1 colony). The narrowest female elytra were 1.96 mm, and the narrowest male elytra were 1.90 mm (both were from the NW I F_1 colony).

Differences between F_1 *colonies from different locations of the same population: field-influenced parental effects*

The different natal fields of F_0 *D. v. virgifera* from the same genetic population (i.e., CSE European population) affected the average of phenotypic trait levels in the subsequently laboratory-reared F_1 colonies for 7 out of 15 traits (47%) (tables 2–6). This field-influenced parental effect was mainly apparent for fitness traits (e.g., 70- and 98-day fecundity, adult life span), and to some extent for morphometric traits (elytron length, head capsule width, and hind tibia length) (table 2). The only activity trait that differed between F_1 colonies was their proportion of adults flying (14% difference between the two tested colonies, table 2).

The environmental conditions in different natal fields of a colony from the same genetic population (i.e., CSE) affected the overall phenotypic variability of the subsequently laboratory-reared F_1 (i.e., overall *CV* of 0.38 for colony CSE1 versus 0.49 for colony CSE2, related samples sign test $P = 0.006$, table 2). The variability of more than half of the examined traits (10 out of 15; 67%) was affected by the original environmental conditions of the natal field (tables 2 and 6). This effect was apparent for most morphometric traits (adult fresh body weight, elytron length, pronotum length and width, and hind tibia length) as well as fitness traits (70- and 98-day fecundity, egg overwintering survival, and adult life span) (table 2). Except for the proportion of adults flying, natal fields had little effect on the variability of activity traits.

Differences between the F_1 *and* F_2 *generation of the same population: effects of early generation laboratory rearing*

F_2 generation *D. v. virgifera* from the NW Italian population differed from the corresponding F_1 of the same colony in the average levels of 5 out of 9 (56%) of the phenotypic traits (tables 3 and 6). Effects of such early generation laboratory rearing were mainly apparent for morphometric traits, (i.e., the F_2 adults were usually slightly lighter, but with slightly longer and wider elytra than their F_1). Effects were also apparent for fitness traits (i.e., the F_2 adults usually laid more eggs, and their eggs overwintered more successfully than the F_1). No such effects were apparent among activity traits.

Early generation laboratory rearing did not affect the overall phenotypic variability of the colony (i.e., overall *CV* of 0.52 of F_1 versus 0.42 of F_2, related samples sign test $P = 0.453$, table 3). The variability of only three out of eight phenotypic traits (38%) was significantly affected (tables 3 and 6). For example, F_2 and F_1 adults had similar variability in their fecundity and adult life span, but the F_2 were less variable in their egg overwintering survival than the F_1 (table 3). F_2 and F_1 adults also had similar variability in their flight take-off response, but the F_2 adults were more variable in the proportion of adults flying than the F_1. F_2 and F_1 adults had similar variability in their elytra lengths and widths, but the F_2 adults were more variable in their fresh body weight than the F_1.

Differences between different generations of the same population: effects of long-term laboratory rearing

The generation number of laboratory reared *D. v. virgifera* colonies (i.e., F_8, F_{23}, and F_{191} USA populations) affected the average levels of 9 out of 15 (60%) measured phenotypic traits (tables 4 and 6). Long-term rearing mainly affected morphometrics. For example, F_{191} adults generally appeared larger and heavier than the F_8 and F_{23} adults (i.e., fresh body weight, elytra length and width, pronotum width, head capsule width, and hind tibia length, table 4). Some differences between F_8 and F_{23} were also detected, but seem not to depend on generation numbers. Long-term rearing also significantly affected some fitness and activity traits. For example, F_{191} adults generally appeared to have a greater 98-day fecundity than the F_{23} adults (703 ± 488, range 0–1814, versus 537 ± 390, range 0–1438). But F_{191} adults were less likely to fly and were slower to initiate flight than the F_{23} and F_8 adults.

In contrast to effects on average levels of traits, long-term rearing usually did not affect the variability of traits. Only 3 out of 14 (21%) traits had differences in overall phenotypic *CV*s (Friedman's two-way ANOVA by ranks $P > 0.05$, tables 4 and 6). Moreover, the slight increase in variability in the proportion of adults flying with increasing generation number is a result of fewer adults flying among longer-reared *D. v. virgifera* colonies.

Differences between colonies from genetically different populations

The three populations with different origins (i.e., NW Italian, CSE European, and USA) differed in the average levels of five out of nine (55%) of the phenotypic traits (tables 5 and 6). The population origin mainly affected the morphometrics. For example, the CSE European adults were usually slightly heavier and larger than the adults of the other populations (table 5). Population origin had little effect on fitness and activity traits. For example, the NW Italian eggs seemed to more successfully overwinter than the USA eggs, and both overwintered more successfully than eggs of the CSE European population. Moreover, the NW Italian adults took off slightly faster than other adults, there was no take-off difference between CSE European and USA adults.

The three genetic populations differed in the variability of five out of eight traits (63%) (tables 5 and 6). This was particularly true for activity traits, to some extent for morphometric traits, but less obvious for fitness traits (table 5). Considering that the USA population was the genetically most variable among the three tested populations, it had surprisingly low phenotypic variability. The USA population only appeared more variable in their egg overwintering survival and activity traits than the European populations. Consequently, no significant effect of a populations' original genetics was detected on the overall phenotypic variability of their laboratory colonies (i.e., differences in overall phenotypic *CV*s; Friedman's two-way ANOVA by ranks $P > 0.05$, table 5).

Discussion

There is a debate among researchers as to whether laboratory bioassays and comparative behavioural studies should be conducted with insects directly collected from the field or with insects reared over several generations under standardized laboratory conditions (Richerson & Cameron,

Table 6. *Summary of effects* of the original natal field, laboratory rearing, and the neutral genetic characteristics of the populations at the origin on averages (Av.) and variability (Var.) of phenotypic traits of young immature adults of *Diabrotica v. virgifera* based on significant differences shown in tables 2 to 5: + = significant effect at $P < 0.05$, ++ = significant effect at $P < 0.005$, 0 = no significant difference, empty = no data. With the exception of fecundity, only pooled data for both sexes is considered.

Effects		Fitness traits					Initial activity traits				Initial morphometric traits						
		Fecundity 70 days	Fecundity 98 days	Egg overwintering survival	Egg-to-adult survival	Adult lifespan	Proportion of adults flying	Flight take - off response	Proportion of adults crawling	Adult crawling speed	Adult fresh body weight	Elytron length	Elytra width	Pronotum length	Pronotum width	Head capsule width	Hind tibia length
Field-influenced parental effect[1]	Av.	+	+	0		+	+	0	0	0	0	+	0	0	0	++	++
	Var.	++	+	++		++	+	0	0	0	+	+	0	+	++	0	+
Effects of early generation laboratory rearing[2]	Av.	+		++	0	0	0	0			++	+	++				
	Var.	0		+		0	+	0			+	0	0				
Effects of long-term laboratory rearing[3]	Av.	0	+	0		0	+	+	0	0	++	+	++	0	++	++	++
	Var.	0	0	+		0	+	+		0	0	0	0	0	0	0	0
Differences between colonies from genetically different populations[4]	Av.	0		++	0	0	0	+			++	++	++				
	Var.	0		+		0	+	++			0	+	+				

[1] Based on table 2.
[2] Based on table 3.
[3] Based on table 4.
[4] Based on table 5.

1974; Prokopy *et al*., 1975; Rossler, 1975; Bush *et al*., 1976; Huettel, 1976; Boiler & Chambers, 1977; Chambers, 1977; Diamond *et al*., 2010). Our study shows that indeed nearly all of 16 tested phenotypic traits of seven different colonies of the maize pest *D. v. virgifera* can vary depending on the natal field of the collected specimens, on the genetic population as well as on short- and long-term laboratory rearing. The advantage of field collected insects might be that they had not adapted to certain laboratory conditions (Rossler, 1975). In fact, the variability inherent in field populations due to field conditions is why studies with pest insects from the field, such as *D. v. virgifera*, are so very relevant. If our intent is to understand the characteristics and vulnerabilities of the insects that plague our crops, the place to seek answers is among the pests *in situ*. However, possible differences originating from the natal environmental field conditions may hinder comparative studies (Diamond *et al*., 2010), something that our results show to be considered also for *D. v. virgifera*. As for *D. v. virgifera*, researchers could use and refer to a number of colonies reared in different laboratories (Lefko *et al*., 2008) or could easily collect specimens from maize fields. Therefore, we have compared a number of different phenotypic traits among seven different *D. v. virgifera* colonies. However, the colonies and the combinations we used for comparisons were not comprehensive. There are many more available colonies maintained at a relatively large number of existing rearing facilities (e.g., USDA-ARS NCARL, Brookings, South Dakota, USA; Biotest laboratories, Sagerheide, Germany; French Agricultural Research, Lamberton, MN, USA; CABI, Delemont, Switzerland and Hodmezovasarhely, Hungary; and others), and the options for field-collected *D. v. virgifera* populations are only limited by the number of heavily infested maize fields.

Many phenotypic traits are sensitive to conditions in the field environment. For example, fitness and activity of *D. v. virgifera* are affected by recent diet experience (Levine *et al*., 2002; Mabry *et al*., 2004). Differences in weather conditions such as temperature can increase or decrease developmental time and influence survival (Toepfer *et al*., 2005). Biological attributes such as insect age also affect phenotypic traits (Li *et al*., 2009). Therefore, in our study, all of the *D. v. virgifera* were tested at a similar age, and were provided with the same type and amount of food. They were reared under similar laboratory conditions based on George & Ortman (1965) and Branson *et al*. (1975). Still, there remains the possibility that slight variation in the controlled laboratory environment or in the quality of food might have occurred from one experimental series to the next. However, due to the large samples size and well-controlled experimental conditions used, we hope that our results can provide good indications about constancies or possible changes in traits that may occur due to influences from field conditions or laboratory-rearing. Our study stands out among other comparisons of populations because it focuses on quantities that might be more tangible than the genes and alleles that are usually the metric for comparing populations.

Differences between F_1 *colonies from different locations of the same population: field-influenced parental effects*

While insects from the field may have the advantage that they have not been selected for adaptations to a variety of laboratory conditions (e.g., *Bactrocera* spp.,. Miyatake & Yamagishi, 1999), they may differ from each other in traits due to variation in local field conditions. Indeed, our study revealed that the original and natal collection site of F_0 – *D. v. virgifera* can affect the average levels and the variability of phenotypic traits in the subsequently laboratory-reared F_1 *ca*. 47 and 67% of the time, respectively (= parental or transgenerational effects). As the tested insects for these research questions originated from the same genetic population (i.e., the CSE European population), a genetic population effect can be excluded. Environmentally driven parental effects were mainly apparent in fitness and morphometric traits. Effects on fecundity might need to be specifically considered when conducting studies related to *D. v. virgifera* fitness. In contrast, averages as well as the variability in activity traits, remained largely stable from F_0 to F_1. Flying and crawling were behavioural phenotypic traits that varied little in our assays; perhaps behaviour is generally less influenced by field origin than fitness or morphometric traits. Whether this is indeed true for specific agriculture-relevant behavioural traits such as host plant finding, mating, and oviposition behaviour remains to be tested.

Differences between the F_1 *and* F_2 *generation of the same population: effects of early generation laboratory rearing*

In general for comparative studies, we advise laboratory rearing of field-collected *D. v. virgifera* until at least the F_2 generation to avoid the varying field-influenced parental effects on the fitness and morphometrics occurring in the F_0 or F_1. Indeed, our results showed that such rearing can affect the average levels and variability of phenotypic traits in 56 and 38% of cases, respectively. This was particularly obvious in a change in morphometrics and in an increase in fecundity and egg survival from F_1 to F_2, but less important for other fitness traits, or for activity traits. Interestingly, Lefko *et al*. (2008) also reported an increased egg survival as well as generational survival in early generations of the laboratory rearing of *D. v. virgifera* (i.e., during rearing from F_0 to approximately F_4 or F_5). It is unclear why some morphological traits changed in our study from F_1 to F_2; however, an increase in fitness characteristics can be understood. For example, a greater fecundity can result from more optimal larvae rearing conditions and subsequent adults for the F_1 (laying F_2 eggs) than for the field grown larvae and adults of the F_0 generation (laying F_1 eggs). From other insects it is also known that laboratory-reared colonies might mature more rapidly or have an increased reproductive rate (Miyatake & Yamagishi, 1999). Therefore, laboratory colonies of *D. v. virgifera* reared over different numbers of generations may be comparable under similar conditions, as in other insects (Rossler, 1975; Diamond *et al*., 2010).

Differences between different generations of the same population: effects of long-term laboratory rearing

Insects in laboratory for long periods may undergo further changes due to certain selections (Richerson & Cameron, 1974; Prokopy *et al*., 1975; Bush *et al*., 1976; Huettel, 1976; Boiler & Chambers, 1977; Chambers, 1977; Hill & Caballero, 1992; Stearns, 1992; Miyatake & Yamagishi, 1999; Scannapieco *et al*., 2009; Khazaeli & Curtsinger, 2010; Spurgeon, 2012), which may also affect the results of studies and hinder comparability. Some insects are known to have experienced drastic changes in both phenotype and genetics, when reared under artificial laboratory conditions (Rossler, 1975). One would expect that

mass rearing of *D. v. virgifera* would select for higher reproductive rates (Mohaghegh *et al.*, 1999), an effect that was only confirmed in the present study through the higher 98-day fecundity in the F_{191} generation versus an earlier generation (e.g., F_{23}) but not for 70-day fecundity – a standard often used for *D. v. virgifera* (Li *et al.*, 2009, 2010; Toepfer *et al.*, 2012). Lefko *et al.* (2008) also found no consistent pattern of increased fecundity or fertility when rearing different laboratory colonies of *D. v. virgifera* up to F_{11}. From other insects it is known that colony fitness can strongly change due to genetic laboratory adaptation, and some may become so differentiated that reproductive barriers arise between them and wild populations (Rossler, 1975). Extreme differentiation has not been reported for *D. v. virgifera*. Even the non-diapause colony can still be easily crossed with wild *D. v. virgifera* after decades in culture (Lefko *et al.*, 2008; Oswald *et al.*, 2011).

In contrast to the relatively minor impacts on fitness, long-term rearing clearly reduced *D. v. virgifera* activity, and slightly increased body size and weight. Both types of traits are correlated (activity negatively with weight, activity positively with pronotum size and other morphometric characters, Li *et al.*, 2010). Therefore, it is not clear whether the selection was on activity traits or on body weight-size or on both. Certainly the typical cages used for adult *D. v. virgifera* mass rearing do not allow or reward much flight, as they are usually of less than 0.5 m^3 size, and cages contain several hundreds of adults (George & Ortman, 1965; Branson *et al.*, 1975). Moreover, there is little need to fly or crawl as food and sexual partners are always close to colony individuals. As above, flying and crawling are behavioural phenotypic traits, which might suggest that other behavioural traits could also be influenced by long-term rearing. In fact, conditions of rearing present few opportunities for any penalty for poor choices by the insect. Care should be taken when individuals from long-term reared laboratory colonies are used for behavioural studies (Richerson & Cameron, 1974; Prokopy *et al.*, 1975; Bush *et al.*, 1976; Huettel, 1976; Boiler & Chambers, 1977; Chambers, 1977). This caution should be extended to analyses of host plant acceptability/recognition or pheromonial responses (Tingey, 1986; Masson *et al.*, 1987), topics that remain somewhat un-investigated for *D. v. virgifera*.

In contrast to the changes in average levels of some traits reported herein, long-term rearing did little to reduce the variability of traits, particularly not to morphometric variability. In contrast, Lefko *et al.* (2008) reported a marginal decline in overall phenotypic variation among laboratory-reared *D. v. virgifera* colonies, but the patterns were often inconsistent. Phenotypic variability could be expected to decrease because (i) environmental variability decreases under constant laboratory conditions and (ii) because multiple bottlenecks and inbreeding during laboratory rearing over many generations reduces genetic variability. Such genetic alterations could include the loss of alleles, decreases in heterozygosity, and shifts in allele frequencies (Masson *et al.*, 1987; Norris *et al.*, 2001). Kim *et al.* (2007) investigated the genetic variability of the USDA-ARS NCARL non-diapause colony of *D. v. virgifera* (>190 generations), versus several diapause colonies (>22, and 3rd–8th generations), and versus wild populations. Astonishingly, the genetic variability (allele richness, expected and/or observed heterozygosity) was found to be similar among most of the diapause laboratory colonies as well as wild populations (Kim *et al.*, 2007). This is likely because several hundred males and females are usually reared per generation (Chad Nielson, 2008, pers. commun.) in the laboratory, which may correspond to some hundreds of effective breeders. Only the non-diapause colony (over 190 generations in colony) showed a moderate (15–39%) loss in genetic variability. There was little evidence that the laboratory colonies of *D. v. virgifera* had undergone significant genetic bottlenecks or selection processes (Kim *et al.*, 2007), as one would expect from other insects (Rossler, 1975; Diamond *et al.*, 2010).

Differences between colonies from genetically different populations

The origins of the genetic populations studied here (CSE European, NW Italian, Central-to-Northern USA) affected the average levels and variability of some phenotypic traits. Such effects of a population's origin are easier to explain than changes due to laboratory rearing. This is because CSE European and NW Italian populations are both expanding populations that originated from two independent introductions from the USA (Miller *et al.*, 2005; Ciosi *et al.*, 2008). During the separate introductions, multiple independent genetic bottlenecks occurred because of multiple independent founder effects (Facon *et al.*, 2006). This resulted in the partition of the genetic variability of the source population (i.e., USA), among the invasive populations that are thus genetically differentiated (Ciosi *et al.*, 2008), which is consequently also reflected in some of their phenotypic traits.

Conclusions

In summary, none of the 16 tested traits, with the possible exception of crawling behaviours, appeared to be consistently stable across tested colonies. All the traits were either influenced by the genetic characteristics of the original population, the original conditions in the collection field and/or the subsequent laboratory rearing. Our data suggest that activity can be studied with field-collected *D. v. virgifera* as well as with colonies reared in laboratory for a few generations, whereas the use of such insects is less advised for comparative research about fitness or morphometrics (table 6). Care should be taken when individuals from long-term reared laboratory colonies are used for behavioural studies. Average levels of morphometric and fitness traits, however, seem to be always prone to change due to rearing. However; the variability in those traits remains relatively stable if enough specimens are used in rearing cycles.

In conclusion, these results should only be used as guidelines. They 'only' provide estimations about the potential for changes in traits that may occur due to influences from field conditions or during laboratory-rearing. There is still much that can be done and learned by comparing populations at the organismal level. Nevertheless, we believe that these results can help researchers determine whether their planned investigations of *D. v. virgifera* fitness, activity, or morphometrics should be conducted with *D. v. virgifera* from laboratory colonies or with field-collected specimens.

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