RESEARCH ARTICLE

Spatial and density-dependent multilevel selection on weed-infested maize

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Abstract Artificial group selection has long been proposed as a useful method for crop breeding, yet the possibility that group selection occurs naturally in agroecosystems has not been explored. Due to natural and/or artificial selection, the fitness of an individual can depend on both the individual's traits, the traits of neighboring individuals, and on group's emergent traits (as density). This process is defined as multilevel selection type I. Using contextual analysis, I detected significant multilevel selection type I at the individual and group levels on weed-infested maize (*Zea mays* L.) genotypes with different spatial patterns and densities. In general, uniformity promoted multilevel selection type I, but this response varied within maize varieties and years. The results herein presented show that crop productivity and weed suppression increased at high density-uniformity conditions, but this also depends on variety. Multilevel selection type II is defined as the differential reproduction of entire groups, a process that in agronomic settings is implemented by humans. A reduced phenotypic variation in the angle of insertion of the oldest living leaf at harvest was identified as a group attribute to be selected for artificial multilevel selection type II. This trait experienced stabilizing selection at the individual level, which significantly increased crop productivity.

Keywords Artificial selection · Contextual analysis · Crop-weed competition · Density-dependence · Multilevel selection · Spatial uniformity

Introduction

Two of the main architects of the Green Revolution, C.M. Donald and J.L. Harper, argued that the selection of entire crop groups should be the strategy implemented in agronomic breeding programs instead of the traditional selection of more-fit individuals (Harper 1977; Donald 1968, 1981). Harper suggested that "…group selection, which is believed to be extremely rare in or absent in nature … may be the most proper type of selection for improving the productivity of crop and forest plants" (Harper 1977; p. 892). Currently, 'group selection' is better understood as multilevel selection, which occurs when natural selection acts simultaneously on two or more levels of the biological hierarchy (Heisler and Damuth 1987; Marín 2016). Donald's concepts of "ideotype" and "communal plant" reaffirmed the possible role of multilevel selection in agroecosystems: "…a

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successful crop ideotype will be a weak competitor … [so] … plants in the crop community will compete with each other to a minimum degree" (Donald 1968); "a communal plant has features in accord with the success of the crop community rather than the plant itself'" (Donald 1981). "Strain selection" was the term used by Donald to describe the process of selecting fitter groups, instead of individuals. This approach has been taken further and has proven to be successful in poultry (Muir 1996) and in crop-weed competition experiments (Weiner et al. 2010).

Weiner et al. (2010) has suggested that high density and spatial uniformity may increase the opportunity for group selection to operate while grain yield by area (productivity) and weed suppression are increased. At high densities, intraspecific competition is lower in an uniform pattern than in the standard clumped pattern implemented in many crop systems, especially cereals -as cereals usually have a very high germination rate advantage over weeds. These conditions can also increase the degree of interspecific competition between crops and weeds (Weiner et al. 2010; Marín and Weiner 2014). In wheat, high density and uniformity growth conditions have significantly increased productivity and weed suppression (Weiner et al. 2001, 2010; Olsen et al. 2005a, b, 2006, 2012). High density planting with narrower bed rows (but not with complete uniformity) has also increased productivity and decreased weed biomass by area in maize (Teasdale 1995, 1998; Murphy et al. 1996; Acciaressi and Chidichimo 2007; Mashingaidze et al. 2009; Mohammadi et al. 2012). A maize study has shown the combined effects of highly dense and uniform growth conditions on increasing grain yield and weed suppression, where the response of grain yield and weed biomass was different for the different genotypes tested (Marín and Weiner 2014).

The fact that reduced intraspecific competition and increased interspecific competition are caused by high-density and uniformity growth conditions, is not necessarily the confirmation of multilevel selection. These effects could be just a response of different survival strategies of individuals, with no multilevel selection operating. To argue that multilevel selection is occurring, the effect of the neighboring individuals' traits on individual fitness must be quantified. In other words, when traits of interacting individuals have an effect on the fitness of a focal individual (Wolf et al. 1999), multilevel selection is occurring. Thus, in the

context of multilevel selection theory, the fitness of an individual depends on its own characteristics and the characteristics of the group to which it belongs. Other concepts of multilevel selection refer to the reproduction of entire groups, which can be directly measured (Goodnight 1985; Heisler and Damuth 1987; Goodnight et al. 1992; Stevens et al. 1995): more fit individuals that exist within less fit groups are less likely to leave more descendants than are individuals with less fitness that exist in more fit groups (Okasha 2006; Traulsen and Nowak 2006; Wilson and Wilson 2007; Simon et al. 2013; Shishi 2014). Two classical definitions of multilevel selection arises from these concepts (Damuth and Heisler 1988; Okasha 2006): multilevel selection type I, which is defined as how much the fitness of an individual depends on the characteristics of the group to which it belongs, and multilevel selection type II, which is the differential reproduction or extinction of entire groups.

Traditionally, quantitative genetics addresses processes regarding multilevel selection type I. To measure the effects of group membership on individual fitness, contextual analysis has been developed (Heisler and Damuth 1987; Goodnight et al. 1992). This method is an extension of the traditional regression method for measuring natural selection (Lande and Arnold 1983) by allowing the inclusion of group characteristics, and it is compatible with "rival" methods such as the direct fitness approach (Goodnight 2013). By employing multiple regressions to determine the effect of individual, mean, and emergent traits on relative fitness, contextual analysis partitions the strength of group and individual selection (Heisler and Damuth 1987; Goodnight et al. 1992). Relative fitness is the reproductive success of a genotype (or phenotype) relative to the maximum reproductive success of the population. When the regression coefficient of a mean trait -average trait value of surrounding individuals- or of an emergent trait -traits only measurable at the group level, as density- are significant, group selection is determined to be occurring. Thus, the fitness of an individual is a function of the characteristics of the group to which it belongs.

Contextual analysis has made possible to detect multilevel selection type I in natural populations of animals (Tsuji 1995; Banschbach and Herbers 1996; Eldakar et al. 2010; Formica et al. 2011; Laiolo and Obeso 2012; Moorad 2013; Searcy et al. 2014;

Campobello et al. 2015) and plants (Stevens et al. 1995; Campbell et al. 1997; Solis et al. 2002; Donohue 2003, 2004; Aspi et al. 2003; Weinig et al. 2007). Multilevel selection type II is more difficult to measure in nature, as it is not easy to trace a group' trajectory and limits, but this type of selection has been demonstrated to exist in controlled laboratory conditions (Goodnight 1985) and even by studying entire communities (Goodnight and Stevens 1997) or ecosystems (Swenson et al. 2000). In agronomic settings, there has been no measure of multilevel selection type I of any kind; the 'group' refers to specific strains selected or bred over time. Natural and artificial multilevel selection can simultaneously act on crops, and the strength and direction of selection can be variable. Donald and Harper (Harper 1977; Donald 1968, 1981) as well as Muir (1996) have demonstrated the existence of multilevel selection type II processes in agricultural systems, but in these studies, humans are referred to as the selective force acting on the crop by purposely selecting group attributes that lead to higher productivity. This is not the same to determining if the average trait value of surrounding individuals affects the fitness of a focal individual-multilevel selection type I.

Which group attributes should be selected at the group level in crops?—multilevel selection type II. Reduced phenotypic variation under high dense-uniform conditions is an emergent trait -the variation among individuals is only measurable at the group level, that could be selected by humans in order to increase productivity and reduce weed biomass (Weiner et al. 2010). Reduced phenotypic variation leads to less canopy interference between crop individuals. So far, density has been the only emergent trait used in contextual analysis though there are other emergent factors that could affect natural selection (Stevens et al. 1995; Campbell et al. 1997; Solis et al. 2002; Aspi et al. 2003; Donohue 2003, 2004; Weinig et al. 2007). As Donohue (2004) suggests, natural selection at any level, especially in sessile organisms, also depends largely on spatial arrangement. Despite this, the effects of spatial arrangement on natural selection have been poorly investigated. Interactions occurring at high density have different fitness effects if the distribution pattern of the individuals interacting is clumped, random, or regular. The competitive ability of a plant depends on its individual characteristics, on the density and spatial pattern in which it

grows (Weiner et al. 2010), and, according to multi-level selection theory, on the the average trait value of surrounding individuals. It is expected that individuals growing in high-density patches have lower mean relative fitness than individuals growing in low-density areas. As such, higher grain yields are expected at high-density patches (Weiner et al. 2010).

This study aimed to: (i) identify the effects of crop density and spatial pattern on the productivity of different maize genotypes and on weed suppression, (ii) identify if processes of multilevel selection type I - where relative fitness is a function of individual, conspecifics', and emergent traits, are occurring on weed-infested maize and to test the effects of density and spatial pattern on those processes, and (iii) to detect which traits show a reduction in phenotypic variation, as a group attribute to be selected, and how density and spatial pattern might affect those traits.

Materials and methods

Experimental design

The experiment was carried for six months, starting in 1 June 2013 and repeated on 2 June 2014 on a farm in the El Tambo municipality, Cauca region, Colombia ($2°25'45.90''$ N, $76°43'34.68''$ W), at 1730 m.a.s.l. Maize was the preceding crop in the study site. The soil was characterized as being a sandy clay inceptisol, with an organic matter content of 3%. Poultry manure fertilizer (*Gallinaza*, Productora Avícola de Occidente S.A.S®, Carrera 2C No. 30-03, Cali, Colombia; 58% OM, 4% N, 2.60% P, 2.30% K) was applied at a rate of 40 kg N ha^{-1} for 8 days before and 1 month after sowing maize seeds. The highest registered temperature for the sowing season in 2013 was 31.3 °C, the minimum was 14.8 °C, and there was an average temperature of 19.6 °C $\pm$ 3.2 °C. The mean monthly precipitation was 150 mm $\pm$ 17 mm. During the 2014 season, the highest, minimum, and average temperatures were 29.9 °C, 15.0 °C, and 18.8 °C $\pm$ 5.7 °C, respectively, and the mean monthly precipitation was 178 mm $\pm$ 28 mm. Three sowing densities (5, 7, and 10.5 seeds m^{-2}), two spatial patterns (grid and rows; inter and intra-row proportion distances were 1:1 for the grid pattern, and approximately 5:1 in the row pattern), and three varieties of maize (*Novillero, Amarillo ICA V-305,* and *Hıbrido HR Oro-Amarillo*)

were used. Thus, a total of 3 densities × 2 spatial patterns × 3 varieties, for a total of 18 treatment combinations replicated 3 times (6 m × 6 m plot each replicate) in a randomized complete block design were tested. Each year there were a total of 54 plots (1944 m² sown). All maize varieties used in this study are cultivated in the region. *Novillero* is a traditional variety, while Amarillo *ICA V-305* and *Híbrido HR Oro-Amarillo* have been recently developed (SemillasArroyave®, Calle 162 No. 18A-32, Bogotá, Colombia). *Híbrido HR Oro-Amarillo* matures more quickly than the other varieties.

All seeds were sown to a depth of 5 cm with a seeding machine; conventional mechanized and intensive tillage practices (including seedbed) were implemented. To obtain high weed pressure, *Brachiaria brizantha* Hochst. Ex A. Rich. (bread grass) seeds (Semicol S. A®, Calle 34 No. 19–38, Bogotá, Colombia) were manually and semi-randomly (thus, reaching the whole plot) sown immediately after maize, at a rate of 30 seeds m⁻². *Brachiaria brizantha* is an invasive and aggressive weed in the region of study. I measured weed biomass, including spontaneous weeds (11% of weed biomass *Brachiaria* sp., 9% *Pteridiumaquilinum* L. Kuhn (bracken fern), 7% *Dichondramicrantha* Urban. (kidney weed), and 5% other species) different from *B. brizantha* (68% of weed biomass), at harvest, which for *Novillero* and *Amarillo ICA V-305* was 180 days (28 November in 2013 and 29 November in 2014), and for *Híbrido HR Oro-Amarillo* was 120 days (29 September in 2013 and 30 September in 2014). All aboveground weed biomass within each plot (6 m × 6 m) was cut at the soil surface at harvest. This biomass was then dried for 48 h at 70 °C and weighed. Total grain yield was manually collected in each plot.

Individual phenotypic traits measured

To measure the effects of mean and emergent traits on relative fitness (multilevel selection type I), 10 neighboring maize individuals growing at the approximate center of each plot were selected; a total of 540 individuals were measured each year. For both the grid and row patterns, the 10 individuals were located in the three central rows. For each plant, individual grain yield was used as a proxy of individual fitness. Relative fitness was calculated as the individual grain yield of each plant relative to the average of each

variety at each year. For the same 10 individuals of each plot, four phenotypic traits were measured: the angle of insertion of the oldest living (i.e. green and not withered) leaf at harvest -which was the leaf number six for all individuals, the height of insertion of the six leaf, the height of the maize stem, and the vegetative biomass -i.e. aerial biomass including stem and leaves. These four traits are strongly related to vegetative growth and to intra and interspecific competition for light (Anten and Vermeulen 2016). The angle of insertion of the oldest living leaf is a trait highly important in terms of intra and interspecific competition for light, especially given its ecophysiological significance regarding competition with weeds (Marín and Weiner 2014), and was measured using photographs of the leaf bases taken perpendicularly to the plane formed by the leaf base and the stem. The photographs were analyzed with the software ImageJ 1.6.0 (Schneider et al. 2012).

Emergent phenotypic traits measured

As suggested by Heisler and Damuth (1987), emergent traits were included in contextual analysis (multiple regression) to detect multilevel selection type I. Emergent traits are those only measurable at the group level. In this study, the emergent traits were density and spatial uniformity. The respective density of each individual was included in the multiple regression, and the Nearest Neighbor Index -NNI (Davis 1986; Hammer 2009) was included as a measure of spatial uniformity. This metric is an average of the distances between a focal individual and every other neighboring individual. Near zero values of the NNI indicate spatial aggregation, and values > 1 indicate uniformity. The NNI was calculated using the coordinates of each pattern and density treatments. R values of the NNI were calculated using the convex hull area calculation and the wrap around edge correction in the software Past 3.12 (Hammer et al. 2001). NNI values for the grid pattern were: 1.6165 (5 seeds m⁻²), 1.6799 (7 seeds m⁻²), and 1.7292 (10.5 seeds m⁻²); for the row pattern the values were: 0.66651 (5 seeds m⁻²), 0.68943 (7 seeds m⁻²), and 0.71152 (10.5 seeds m⁻²).

Statistical analysis

To test the effects of density, pattern, variety, and their interactions on grain yield and weed biomass per m^{-2}, mixed linear models were implemented in RStudio 1.2.5033 (RStudio Team 2019). Variables were checked for normality. The models employed were run using the *lme* function of the R package *nlme* (Pinheiro et al. 2019), based on likelihood principles in which blocks are treated as random effects.

As suggested by Stevens et al. (1995) and Weinig et al. (2007), before performing the contextual analysis, principal component analysis was used to reduce dimensions of the measured phenotypic traits because of the high correlation among the variables. Thus, highly correlated traits could be analyzed as a single factor (underlying or latent variable, ie. "size") in the quantitative genetics analysis of natural selection (Crespi and Bookstein 1989). The R base function *princomp* was used to identify significant principal components. Then, factors that individually explained more than 10% of data variance were selected and rotated using the Varimax method (R function *varimax*), which maximizes data variation by the composite variation (Anderson 1984).

To estimate the strength and direction of multilevel selection type I on the measured phenotypic traits, contextual analysis was performed (Heisler and Damuth 1987; Goodnight et al. 1992). In contextual analysis, the effect of individual traits, ie. the factors detected using the principal component analysis described above, mean traits, i.e. the mean value of the factor of the neighboring individuals excluding the factor of the focal individual, and emergent traits (density and spatial uniformity) on relative fitness are determined using multiple regressions. Thus, each individual has unique values of relative fitness, individual, and mean traits -as each individual is excluded for the calculation of the mean trait. The regression coefficients for the individual traits are interpreted as the strength of individual-level selection, while the regression coefficients for the mean traits are interpreted as the strength of group-level selection; the respective sign of each coefficient gives the direction of selection (Heisler and Damuth 1987). Contextual analysis was also pooled over the two pattern treatments (grid and row pattern). For this study in particular, contextual analyses was explained by the following formula:

$$w = \beta' F1 + \beta' F2 + \beta' \varpi F1 + \beta' \varpi F2 + \beta' Dens. + \beta' Pattern$$

where relative fitness (w) of maize plants depends on the partial regression coefficients (β') of the factors 1 (F1) and 2 (F2), the mean values of neighboring individuals for each factor ($\hat{w}$F1 and $\hat{w}$F2), and the contextual traits density (Dens.) and spatial pattern (Pattern). Factors result from the above-explained principal component analysis.

To identify if phenotypic variation had an effect on maize mean fitness -as a measurement of multilevel selection type II, mixed linear models were implemented in RStudio. These models were run using the *lme* function of the R package *nlme* (Pinheiro et al. 2019), based on likelihood principles in which blocks are treated as random effects. Each model included mean relative fitness as the dependent variable, and the standard deviation of each factor obtained in the principal components analysis and its interactions with density, pattern, and variety as independent variables.

Results

Effects of sowing pattern and density on grain yield and weed suppression

There were strong and highly significant effects of density, pattern, and variety on grain yield and weed biomass in both years (Table 1). Most interactions among these variables were also significant, but the interaction effects were not as strong as the main effects. Comparing plots of the grid pattern at the highest density with those of normal sowing practice (row pattern, medium density), weed biomass was reduced by 93.2%, 76.1%, and 48.6%, in *Novillero*, *Amarillo ICA V-305*, and *Hıbrido HR Oro-Amarillo* in 2013, and 82.7%, 65.6%, and 39.9% in 2014, respectively. For one variety, *Hıbrido HR Oro-Amarillo*, weed biomass decreased only slightly from medium to high density sowing (Fig. 1). The effect of density on grain yield also differed among the three varieties (Table 1). In both years, *Hıbrido HR Oro-Amarillo* decreased in grain yield from medium to high density sowing, whereas grain yield always increased with density and spatial uniformity in the other two variaties (Fig. 1). In comparison to the treatment with

Table 1 Mixed linear model for dry weed biomass and maize grain yield in g m^{-2}

| Variable | Weed Biomass | | | | Grain Yield | | | |
| | 2013 | | 2014 | | 2013 | | 2014 | |
	N df	F	N df	F	N df	F	N df	F
Density	2	977.225***	2	542.209***	2	306.479***	2	436.641***
Pattern	1	3037.130***	1	2705.931***	1	248.780***	1	323.568***
Variety	2	121.542***	2	2.723^{ns}	2	104.911***	2	145.280***
Density * Pattern	2	6.122**	2	4.618**	2	9.373***	2	10.658***
Density * Variety	4	25.851***	4	26.453***	4	53.744***	4	78.327***
Variety * Pattern	2	147.432***	2	97.957***	2	16.869***	2	31.848***
Density * Variety * Pattern	4	11.834***	4	41.679***	4	2.589^{ns}	4	4.096**

In all cases, D df = 36

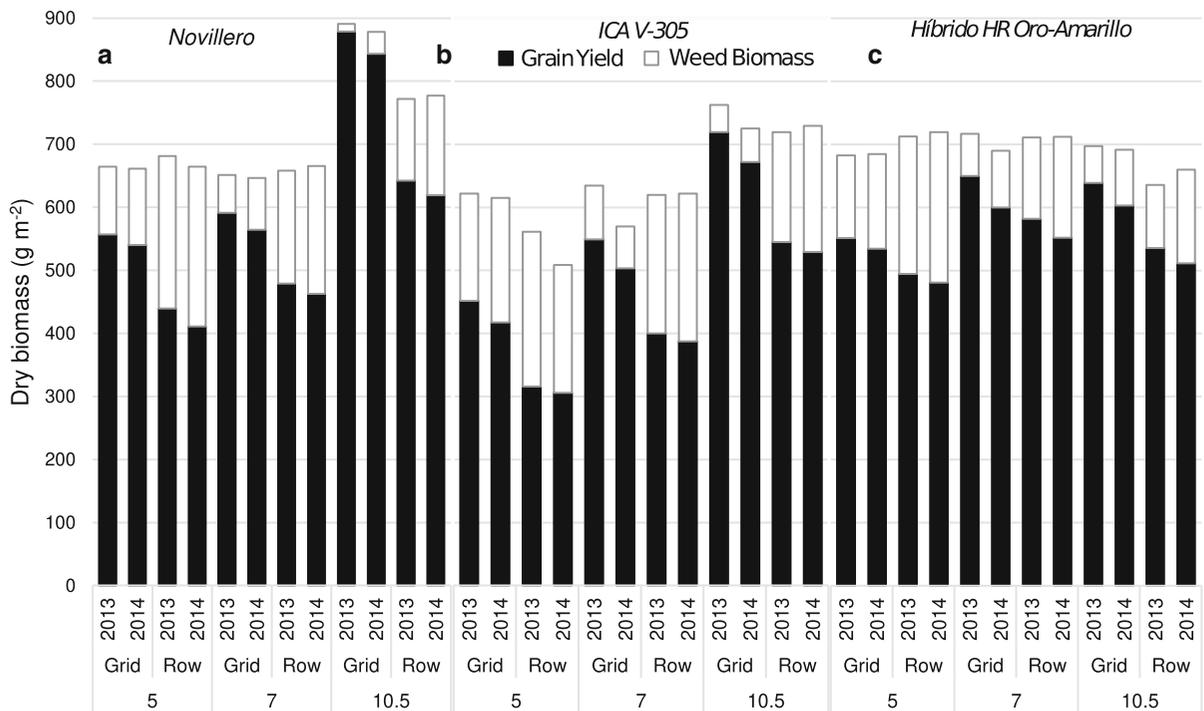$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns non-significant

Fig. 1 Dry biomass (g m^{-2}) of maize grain yield (black bars) and weed biomass (white bars) for the *Novillero* variety (**a**), *ICA V-305* variety (**b**), and *Híbrido HR Oro-Amarillo* (**c**) grown at three densities (5, 7, and 10.5 seeds m^{-2}) and in two spatial patterns (grid and row) in 2013 and 2014

standard practices (row pattern, medium density), the high density-uniform treatment increased the grain yield of *Novillero, Amarillo ICA V-305*, and *Híbrido HR Oro-Amarillo* by 83.5%, 66.7%, and 7.6% in 2013, and 82.4%, 58.0%, and 7.1% in 2014, respectively.

Multilevel selection type I on weed-infested maize

The rotated factor 1 had large positive loadings for the height of insertion of the six leaf, stem height, and vegetative biomass -this factor explained 72.504% of

Table 2 Loadings on principal components for the three maize varieties in both years

Trait/factor	Novillero				ICA V-305				Hıbrido HR Oro-Amarillo			
	2013		2014		2013		2014		2013		2014	
	1	2	1	2	1	2	1	2	1	2	1	2
Angle insertion 1st leaf	− 0.102	*0.995*	− 0.104	*0.995*	− 0.268	*0.963*	0.000	*1.000*	0.035	*0.999*	0.027	*1.000*
Height insertion 1st leaf	*0.941*	− 0.068	*0.941*	− 0.085	*0.935*	0.122	*0.953*	0.019	*0.961*	0.041	*0.966*	0.034
Stem height	*0.980*	− 0.116	*0.981*	− 0.110	*0.984*	0.073	*0.989*	− 0.008	*0.990*	0.036	*0.992*	0.023
Vegetative biomass	*0.980*	− 0.116	*0.981*	− 0.109	*0.984*	0.073	*0.986*	− 0.012	*0.982*	0.026	*0.992*	0.023

Factors with loadings > 0.9 are show in italics due to their dominant role in that factor

Factor 1 ("size") accounted for 72.504% of variance, Factor 2 ("angle) accounted for 23.467% of variance

variance. This factor is referred to as "size" (Table 2). Rotated factor 2 had large positive loadings for the angle of insertion of the six leaf, and is referred as "angle" (Table 2) -this factor explained 23.467% of variance, for a total of 95.971% of variance explained by these two factors. Factors 3 and 4 explained less than 5% of variance in total, and thus were not included.

The effects of individual (factor 1 and factor 2), mean (means of factor 1 and factor 2, excluding the focal individual), and emergent (density and spatial uniformity) traits on relative fitness were different among the three varieties in both years, as measured using contextual analysis (Table 3). Factor 1-size, an individual trait, was the only one having an effect among all varieties in both years. When measured alone, positive directional selection on factor 1-size was found; alternatively, a stabilizing pattern was found for factor 2-angle (Fig. 2). When significant, changes in relative fitness were positively or negatively affected by density and spatial uniformity (Table 3). When both the individual and mean factor regression coefficients were significant, group selection was stronger and acted in the same direction as individual selection (Table 3). This was the case for all varieties and years except for factor 2-angle in the variety *ICA V-305* in 2014 (Table 3). When the treatments were pooled by pattern (grid and row), stronger group selection effects were detected in the grid pattern (Table 4). However, some group selection was detected in the row pattern as well (for *Novillero* and *ICA V-305* in 2013).

Multilevel selection type II and the opportunity for strain selection

As expected, individual and mean fitness were inversely related to density, and higher individual and mean fitness values were found in the grid pattern (Fig. 3). The effect of phenotypic variation, as a group attribute, on mean relative fitness, was dependent on sowing pattern and density (Fig. 4). However, only factor 2-angle, had a significant effect on mean fitness (Table 5). High density and spatial uniformity promoted less variation in factor 1-size and factor 2-angle (Fig. 4).

Discussion

Increased density and spatial uniformity affected each maize genotype differently. The *Novillero* variety had a stronger response to these treatments than did the *Hıbrido HR-Oro Amarillo* variety, as this variety only experienced a slight increase in productivity and weed suppression (Fig. 1). Thus, maize genotypes that have a good competitive ability (*Hıbrido HR-Oro Amarillo*) against weeds at medium growth densities and growing in aggregated patterns (the standard sowing practice) do not necessary respond in the same way at high density and uniformity conditions. But varieties with moderate responses to standard sowing practices (*ICA V-305* and mainly *Novillero*) considerably increased their productivity and weed suppression when grown in higher density-uniform patterns.

Table 3 Contextual analysis for the three maize varieties during both years. Standardized selection coefficients (partial regression coefficients, β') from a contextual analysis on relative fitness performed with individual (factor 1-size and factor 2-angle), mean (means of factor 1 and factor 2, excluding the focal individual), and contextual (density and spatial uniformity) traits are shown as $\beta' \pm SE$ (F-statistic)$^{p\ value}$

Traits	Novillero		ICA V-305		HR Oro-Amarillo	
	2013 $r^2 = 0.777$	2014 $r^2 = 0.660$	2013 $r^2 = 0.589$	2014 $r^2 = 0.566$	2013 $r^2 = 0.641$	2014 $r^2 = 0.539$
Factor 1 (size)	**0.294 ± 0.077** **(3.791)*****	**0.375 ± 0.097** **(3.864)*****	**0.376 ± 0.091** **(4.129)*****	**0.245 ± 0.092** **(2.680)****	**0.396 ± 0.065** **(6.105)*****	**0.288 ± 0.069** **(4.169)*****
Factor 2 (angle)	**0.278 ± 0.049** **(5.693)*****	0.020 ± 0.071 (0.281)^{ns}	− 0.067 ± 0.063 (− 1.066)^{ns}	**0.225 ± 0.074** **(3.040)****	**0.098 ± 0.049** **(2.021)***	0.133 ± 0.073 (1.825)*
Mean factor 1	**0.410 ± 0.160** **(2.925)****	**0.918 ± 0.181** **(5.072)*****	0.420 ± 0.215 (1.947)^{ns}	**0.646 ± 0.213** **(3.029)****	0.228 ± 0.250 (1.269)^{ns}	0.303 ± 0.188 (1.612)^{ns}
Mean factor 2	**0.706 ± 0.259** **(6.841)*****	− 0.195 ± 0.180 (− 1.086)^{ns}	**− 0.245 ± 0.121** **(− 2.027)***	**− 0.578 ± 0.176** **(− 3.285)****	− 0.054 ± 0.217 (− 0.927)^{ns}	− 0.025 ± 0.140 (− 0.178)^{ns}
Density	**− 0.541 ± 0.188** **(− 2.876)****	**0.723 ± 0.276** **(2.625)****	0.302 ± 0.277 (1.089)^{ns}	**0.817 ± 0.303** **(2.693)****	− 0.225 ± 0.191 (− 1.180)^{ns}	− 0.221 ± 0.214 (− 1.033)^{ns}
Spatial uniformity (NNI)	0.007 ± 0.131 (0.109)^{ns}	**0.452 ± 0.200** **(− 4.551)*****	**0.479 ± 0.134** **(7.198)*****	**0.384 ± 0.117** **(6.598)*****	**− 0.178 ± 0.107** **(− 3.371)****	0.106 ± 0.139 (− 1.540)^{ns}

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns non-significant. Significant coefficients in bold
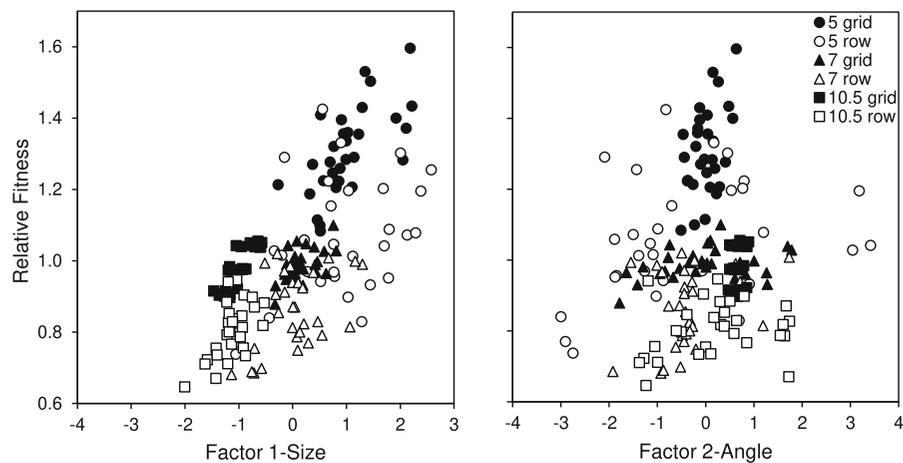

Fig. 2 Relationship of factor 1-size (**a**) and factor 2-angle (**b**) with relative fitness for the maize variety *Novillero* in 2013. The experiment was performed using three sowing densities: 5 seeds m^{-2} (circles), 7 seeds m^{-2} (triangles), and 10.5 seeds m^{-2} (squares); and two spatial patterns: grid pattern (filled symbols) and row pattern (empty symbols). For factor 1-size there was a similar response for the three varieties in both years. For factor 2-angle there was a similar response for *ICA V-305* in 2014 and *Híbrido HR Oro-Amarillo* in 2013

This confirms that the competitive ability and productivity of maize depends on the plants' genotypic characteristics as well on the density and spatial pattern in which they are grown (Marín and Weiner 2014). Thus, the competitive ability is spatially and density dependent; this being something that is contrary to what is usually assumed by weed scientists (Weiner et al. 2010; Marín and Weiner 2014).

The results of this study indicate that in weed-infested maize, the fitness of maize individuals

Table 4 Contextual analysis for the three maize varieties during both years, performed independently for each partial pattern. Standardized selection coefficients (partial regression coefficients, β') from a contextual analysis on relative fitness performed with individual (factor 1-size and factor 2-angle), mean (means of factor 1 and factor 2, excluding the focal individual), and contextual (density) traits are shown as $\beta' \pm SE$ (F-statistic)$^{p\ value}$

Traits	Novillero		ICA V-305		HR Oro-Amarillo	
	2013	2014	2013	2014	2013	2014
> Grid pattern						
	$r^2 = 0.913$	$r^2 = 0.899$	$r^2 = 0.616$	$r^2 = 0.726$	$r^2 = 0.743$	$r^2 = 0.693$
Factor 1 (size)	**0.224 ± 0.101**	0.204 ± 0.125	0.187 ± 0.171	**0.352 ± 0.131**	**0.236 ± 0.111**	**0.473 ± 0.089**
	(2.161)*	(1.698)^{ns}	(1.152)^{ns}	**(2.532)***	**(2.388)***	**(5.363)***
Factor 2 (angle)	**0.263 ± 0.057**	− 0.108 ± 0.223	**− 0.241 ± 0.121**	**0.696 ± 0.346**	0.133 ± 0.072	0.086 ± 0.185
	(6.361)*	(− 1.313)^{ns}	**(− 2.353)***	**(3.228)***	(1.781)^{ns}	(0.836)^{ns}
Mean factor 1	**0.547 ± 0.145**	**2.379 ± 0.167**	0.471 ± 0.273	**1.005 ± 0.208**	**0.766 ± 0.370**	0.254 ± 0.200
	(3.894)*	**(13.305)***	(1.636)^{ns}	**(3.969)***	**(2.760)***	(1.099)^{ns}
Mean factor 2	**0.936 ± 0.202**	**− 3.542 ± 0.347**	**− 1.373 ± 0.296**	− 1.894 ± 0.922	− 0.020 ± 0.478	0.083 ± 0.380
	(12.344)*	**(− 13.192)***	**(− 4.282)***	(− 1.505)^{ns}	(− 0.172)^{ns}	(0.218)^{ns}
Density	**− 0.929 ± 0.141**	**5.366 ± 0.356**	**1.353 ± 0.418**	1.836 ± 1.080	0.109 ± 0.268	− 0.302 ± 0.373
	(− 5.962)*	**(13.991)***	**(0.329)***	(1.305)^{ns}	(0.359)^{ns}	(− 0.719)^{ns}
> Row pattern						
Factor 1 (size)	$r^2 = 0.554$	$r^2 = 0.504$	$r^2 = 0.468$	$r^2 = 0.399$	$r^2 = 0.576$	$r^2 = 0.442$
	0.398 ± 0.108	**0.453 ± 0.117**	**0.527 ± 0.110**	0.241 ± 0.128	**0.462 ± 0.088**	**0.211 ± 0.105**
	(2.966)*	**(3.112)***	**(3.812)***	(1.660)^{ns}	**(4.843)***	**(1.977)ns**
Factor 2 (angle)	**0.236 ± 0.078**	0.059 ± 0.087	− 0.039 ± 0.078	**0.385 ± 0.097**	0.090 ± 0.074	0.171 ± 0.094
	(2.129)*	(0.444)^{ns}	(− 0.384)^{ns}	**(3.043)***	(1.222)^{ns}	(1.502)^{ns}
Mean factor 1	0.530 ± 0.296	**0.657 ± 0.267**	0.378 ± 0.351	**0.790 ± 0.374**	0.118 ± 0.373	0.297 ± 0.326
	(1.755)^{ns}	**(2.143)***	(0.929)^{ns}	**(2.025)***	(0.477)^{ns}	(1.018)^{ns}
Mean factor 2	0.310 ± 0.504	− 0.059 ± 0.244	− 0.008 ± 0.159	− 0.269 ± 0.245	− 0.044 ± 0.293	− 0.035 ± 0.181
	(1.522)^{ns}	(− 0.206)^{ns}	(− 0.049)^{ns}	(− 1.209)^{ns}	(− 0.572)^{ns}	(− 0.209)^{ns}
Density	− 0.094 ± 0.380	0.413 ± 0.444	0.175 ± 0.450	0.773 ± 0.472	− 0.247 ± 0.305	− 0.216 ± 0.340
	(− 0.210)^{ns}	(0.815)^{ns}	(0.341)^{ns}	(1.668)^{ns}	(− 0.881)^{ns}	(− 0.691)^{ns}

$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns non-significant. Significant coefficients in bold

depends both on their individual traits, the mean traits of neighboring individuals, and spatial pattern and density (Tables 3, 4). This is a clear indication of multilevel selection type I. Increased sowing density and uniformity had different effects on individual relative fitness, as the effects were different by variety and year. This indicates that multilevel selection should be studied on a case-by-case basis, as indicated by Goodnight and Stevens (1997) and Wilson and Wilson (2007). However, in most of the treatments of this study, spatial uniformity increased individual relative fitness (Tables 3, 4, Fig. 2). Here I found that in most traits, across varieties and years, individual

and group selection acted in the same direction, but group selection was usually stronger (Table 3). While individual and group selection usually act in different directions (Aspi et al. 2003; Donohue 2003, 2004), this is not a requisite for multilevel selection to operate (Goodnight et al. 1992). Furthermore, here lays the main difference with measurement approaches such as kin selection or direct fitness: measuring direct fitness makes it possible to just identify selection occurring at both levels (individual and relatives) but acting in different directions (Goodnight 2013).

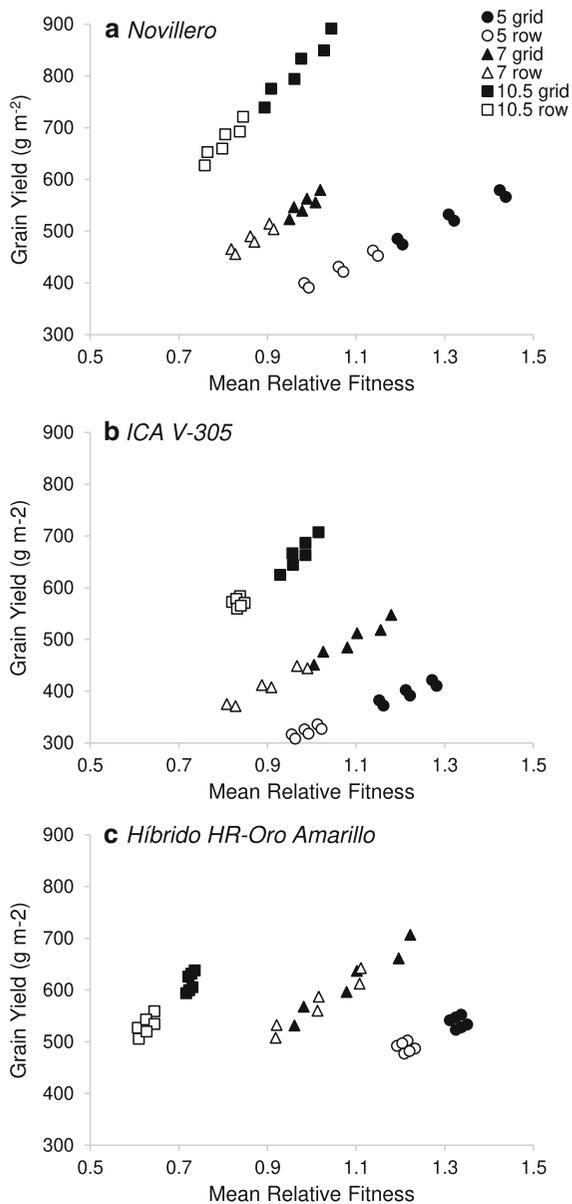Despite the strong evidence of multilevel selection in natural populations (Marín 2016), there still seems

Fig. 3 Relationship between mean relative fitness and maize grain yield (g m^{-2}) for the *Novillero* variety (**a**), the *ICA V-305* variety (**b**), and the *Híbrido HR Oro-Amarillo* variety (**c**) in both years. The experiment was performed at three densities: 5 seeds m^{-2} (circles), 7 seeds m^{-2} (triangles), and 10.5 seeds m^{-2} (squares); and in two spatial patterns: grid pattern (filled symbols) and row pattern (empty symbols)

to be some debate regarding this important process in nature (Wilson and Wilson 2007). In plants, where neighborhood identity has been shown to directly and indirectly affect heritability (Griffing 1989), density and spatial arrangement should be studied in terms of

multilevel selection. While in this study, both density and spatial arrangement were imposed variables, there is no technical difficulty in measuring or plotting both contextual traits in natural populations or meta-populations.

Goodnight and Stevens (1997) specify that multi-level selection type I is variable within and between species and time. Thus, is not possible to establish general patterns within and between species and time because individual and group selection are forces that are constantly changing (Traulsen and Nowak 2006). Furthermore, within varieties of a single species such as maize, group selection on a trait could also differ. Uniform growth conditions can increase the detection of group selection (Donohue 2004). This was found in the present study (Tables 2, 3), but this response to uniform conditions varied among varieties (Tables 2, 3).

The *Híbrido HR Oro-Amarillo* variety had the weakest response to high density and uniformity conditions with respect to weed suppression, productivity, and mean relative fitness (Figs. 1, 3). In this variety, there was also a less significant effect of mean and emergent traits on individual relative fitness (Tables 2, 3). An opposite result was found for the *Novillero* variety. Thus, there seems to be a direct relationship between multilevel selection type I and weed suppression and productivity increasing. Varieties with higher productivity and weed suppression at high density and uniformity conditions were those where multilevel selection type I was mostly detected.

Reduced phenotypic variation in the angle of insertion of the oldest living leaf (six leaf in this study; factor 2-angle) seems to be a good emergent trait to be selected at breeding's programs. In this case, the interaction of variety and phenotypic variation did not affected mean group fitness (Table 5). A reduced phenotypic variation in this trait highly increased mean relative fitness, and this was strongly dependent on density and spatial arrangement (Table 5, Fig. 4). Thus, a grid pattern promotes both higher grain yield by area and higher individual and mean fitness; however, high density promotes higher grain yield by area and lower individual and mean fitness (Fig. 3). At high densities, there are smaller and less variable individuals with lower fitness that produce higher grain yields by area (Fig. 2). Furthermore, this effect is stronger with spatial uniformity at least in two of the three varieties studied (Fig. 1). Angle of insertion
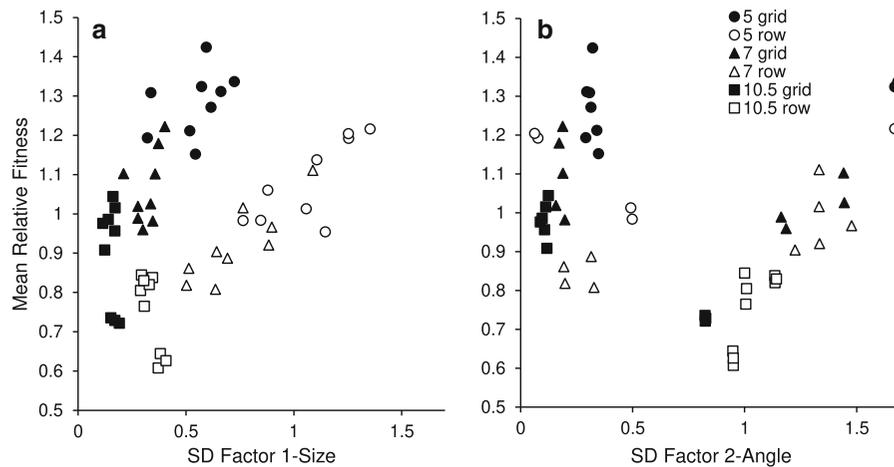
Fig. 4 Relationship of the standard deviations of factor 1-size (**a**) and factor 2-angle (**b**) with mean relative fitness for the three maize varieties in 2013. The experiment was performed at three densities: 5 seeds m^{-2} (circles), 7 seeds m^{-2} (triangles), and

10.5 seeds m^{-2} (squares); and in two spatial patterns: grid pattern (filled symbols) and row pattern (empty symbols). A similar response was found for 2014

Table 5 Mixed linear models of maize mean relative fitness using the standard deviations of the individual factors 1 and 2 (size and angle)

Source	2013			2014	
	N df	F		N df	F
> *Factor1: size*		$r^2 = 0.887$			$r^2 = 0.709$
SD	1	1.243^{ns}		1	3.560^{ns}
SD*Variety	2	1.561^{ns}		2	2.905^{ns}
SD*Pattern	1	30.264***		1	13.424**
SD*Density	2	6.797**		2	25.182***
> *Factor 2: angle*		$r^2 = 0.890$			$r^2 = 0.665$
SD	1	7.637**		1	6.847*
SD*Variety	2	0.115^{ns}		2	1.462^{ns}
SD*Pattern	1	4.554*		1	0.144^{ns}
SD*Density	2	27.535***		2	42.970***

In all cases, D df = 47

$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, *ns* non-significant

(factor 2-angle), which had a significantly reduced phenotypic variation and increased mean fitness (Fig. 4, Table 5), presented a stabilizing pattern at the individual level (Fig. 2). Meanwhile the phenotypic variation of factor 1-size did not affected mean relative fitness (Table 5), and had a directional response (Fig. 2).

Increased density and spatial uniformity constitute crop growth strategies that could be implemented in agriculture to increase productivity and reduce competition with weeds. However, the response to these growth conditions depends on the genotypic characteristics of the plant: not all varieties respond in the same way. Herein it is shown that multilevel selection type I occurs in weed-infested maize, and the response to individual and group selection varies within genotypes and years. In most cases, spatial uniformity increases multilevel selection type I. Reduced variation in the angle of insertion of the oldest living leaf at harvest seems to be an useful plant trait for selective breeding programs. Reduced phenotypic variation in this trait at high density and uniformity growth conditions increases productivity: more individuals with lower relative fitness achieve more grain yield by area.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflicts of interest   The authors declare that they have no conflict of interest.

Human and animal righs   This manuscript does not involve human participants or animals.

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