Allometric analysis of the effects of density on reproductive allocation and Harvest Index in 6 varieties of wheat (*Triticum*)

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ABSTRACT

Plants produce biomass and then allocate some of this biomass to reproduction. The pattern of reproductive allocation is an important aspect of a plant's reproductive strategy in nature and is closely linked to yield and Harvest Index in cereal crops. Recent research has concluded that reproductive allocation should be analyzed and interpreted allometrically because ratios or fractions such as Reproductive Effort or Harvest Index are size dependent. We investigated reproductive allocation of individuals in 6 varieties of *Triticum* (wheat) grown at a wide range of densities. We harvested leaves, stems, spikes and grains of individual plants and analyzed the relationship between grain mass and vegetative mass allometrically. The large variation in density created large variation in plant mass and reproductive output. Most of the variation in individual yield (grain mass) was due to variation in plant size. There were significant differences among the varieties in the allometric exponent (slope of log–log relationship) of grain versus vegetative mass, such that some varieties produced higher yield (and therefore had a higher Harvest Index) than others when plants were small, while others had higher yield at larger sizes. Thus, the Harvest Index and its rank among varieties changed with plant size, which puts into question the practice of selecting for Harvest Index when crop performance varies greatly among individuals, years or environments. Selection for a high Harvest Index when individuals are large may mean unintentional selection for a lower Harvest Index when individuals are smaller. We conclude that cereal breeders should focus on reproductive allometry when interpreting Harvest Index, and select for allometric patterns that are most advantageous in a given agronomic context, especially when there is large variation in productivity among individuals, locations or years.

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1. Introduction

Growth and reproduction are two central aspects of plant life history. Plants produce biomass and then distribute it to different structures, including reproductive structures (Bazzaz and Reekie, 1985; Weiner et al., 2009). Plant species vary greatly in their patterns of reproductive allocation. Some species allocate a large fraction of the resources to reproduction, while other species allocate more resources to structures and functions that increase their ability to compete with other plants, defenses against enemies, or other activities that increase survival and growth. Such differences are the bases for different life-history strategies (Silvertown and Charlesworth, 2001).

While species and ecotypes within a species vary in their allocation patterns, there is also much variation within populations. Some of this variation may be genetic, but much is induced by the biotic and abiotic environment within which the individual plant has grown. Within a population, the most important factor influencing reproductive output is plant size (Weiner, 1988; Sugiyama and Bazzaz, 1998; Méndez and Karlsson, 2004). The size dependence of reproduction has been documented in many studies (Eckersley and Jasienski, 1990; Schmid and Weiner, 1993; Sugiyama and Bazzaz, 1998; Cheplick, 2005). For example, some annual plants have a larger proportion of their biomass in reproductive structures (Reproductive Effort, RE, reproductive mass divided by total aboveground mass) with increasing size, whereas in many perennials species RE decreases with increasing plant size (Weiner et al., 2009). While reproductive allocation has often been described and analyzed in terms of RE, it has been argued that the relationship between plant size and reproductive allocation should be analyzed allometrically, not as ratios such as RE, because such ratios change with size in most cases (Weiner, 2004). Many plants show highly fixed reproductive allometry so the effects of resource levels and

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Abbreviations: RE, Reproductive Effort; HI, harvest index; R, reproductive biomass; V, vegetative (i.e. non-reproductive) biomass.
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competition on reproductive allocation are not direct, but mediated by plant size. Plants grow to the extent that their environment allows, and the size they achieve determines their potential reproductive output (seed production) according to a relatively fixed plan, which is genetically determined.

The study of the allometry of allocation has important implications for plant production systems. Crop grow and produce biomass, and then allocate some of this biomass to "harvestable yield". According to this conceptualization, there are two ways to increase crop yield: (1) increase biomass production, and (2) increase the proportion of this biomass production that is harvestable (HI). Since the harvestable part of biomass is often fruits and seeds, RE in plant ecology and HI in agronomy are closely related. Increased allocation of biomass to reproductive organs accounts for much of the progress in breeding for high yield potential in wheat, oat, barley, maize and sunflower (Slafer, 1994). Agronomic research would benefit from an allometric analysis of yield components, in which the effects of different agricultural practices on biomass production and on harvestable yield are not confounded (Weiner, 2004), rather than the analysis of ratios such as HI (Jasienski and Bazzaz, 1999).

To ask if the Harvest Index is size dependent and if reproductive allometry can contribute to agronomic research, we investigate the effects of density on reproductive allocation in 6 varieties of *Triticum* (wheat). We grew each of these varieties at 4 densities, harvested leaves, stems, spikes and grains of individual plants, and analyzed the relationship between grain production and vegetative biomass allometrically.

2. Materials and methods

2.1. Experimental design

Two diploid (*Triticum monococcum* L.), MO1 and MO4, and two tetraploid (*Triticum dicoccum* Schuebl.), DM22 and DM31, relatives of the modern hexaploid wheat plant, and two modern hexaploid wheat genotypes (*Triticum aestivum* L.), HST and L8139, were investigated. The modern hexaploid cultivars were bred and selected for the semiarid area of the Loess Plateau of China, and all of the genotypes were procured from the Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing. For simplicity, the species/genotypes will be referred to here as genotypes. Field experiments were conducted in 2008 at Yuzhong experimental station of Lanzhou University (104°09′E, 35°56′N, altitude 1750 m). The soil is a loess-like loam, with a bulk density of 1.37 g/cm^3, and a field water-holding capacity of 25% (determined gravimetrically).

The area to be planted was cultivated before sowing to produce a fine seedbed, and 128 kg N, 120 kg P and 15 kg K ha^{-1} was applied before planting. Seeds of each cultivars were sprinkled on 100 cm × 100 cm plots at four densities: 10 (very low), 100 (low), 1000 (medium) and 3000 (high) seeds/m^2. Six cultivars × 4 densities = 24 treatments. There were three replicates for each treatment. The 72 plots were arranged in a completely randomized design in the field with 30 cm between the individual plots. At the early jointing, heading and grain filling stages, the field was irrigated with 50 mm water with flood irrigation controlled by a flow meter. Rainfall during the period from sowing to the last harvest was 229 mm.

Plants were sown on 15 March and harvested on 26 June. The plots were surrounded by supports of wire netting to prevent the plants from lodging, and 25 cm wide buffer zone rows were planted to minimize edge effects. Plants were sprayed with Chlorpyriphos (Dow Agrosciences, USA) against pest attack and streptomycin solution against bacterial diseases. Plots were fertilized every 2 weeks during the 110-day growth period with 500 ml solution containing 1% urea and 0.2% potassium dihydrogen phosphate.

Randomly selected individual plants within a centrally placed 40 cm × 40 cm subplot were harvested at ground level. Twenty to sixty individuals were randomly collected from the three plots at the 3 highest densities, but only 4–5 individuals were harvested in the very low density plots. Plants were put in paper bags individually, dried at 65 °C for 48 h, and weighed. Shoot biomass, spike biomass and grain yield of each plant were weighed separately.

2.2. Statistical methods

Grain yield is the primary aim of cereal production, so we follow agricultural convention and use grain yield as reproductive biomass. It has been argued that analyzing the relationship between reproductive and total biomass can result in a 'spurious correlation' (Brett, 2004), because total biomass includes reproductive biomass. Other researchers have argued that this problem is insoluble or non-existent, since none of the three variables (vegetative, reproductive and total biomass) is independent from the other two (Prairie and Bird, 1989). We analyzed the relationship between reproductive biomass (R) and vegetative (i.e. nonreproductive) biomass (V) of individuals at each density.

Data were log-transformed to homogenize variances. Visual inspection of residual versus predicted y-value confirmed that the residuals were consistent with the assumptions of the analysis. Linear regression was used to determine scaling exponents (slope) and allometric constants (intercept), according to the "allometric equation" (Huxley, 1972):

$$R = \beta V^{\alpha} \tag{1}$$

which is usually analyzed as

$$\log R = \log \beta + \alpha \log V \tag{2}$$

β is often referred to as the "allometric coefficient", log β as the "intercept" and α as the "allometric exponent" in Eq. (1) or the "slope" in Eq. (2). For the full analysis we used a mixed linear model, with log R as the response variable and log density, genotype and log V as variables. Non-significant ($P > 0.1$) interactions were sequentially removed from the analysis (Zar, 1999).

For comparison with traditional agronomic analyses, HI of each genotype at each density was computed at the plot level.

3. Results

The large variation in density created large variation in the size and reproductive output of individuals both within and among the treatments. Mean individual biomass, averaged across all 6 varieties, was 40.41 g at the lowest density and 0.75 g at the highest density.

Most of the variation in log (grain mass) produced by individuals could be explained by variation in log (vegetative mass). In the full data set (Fig. S1 in Supplementary Data), the log R–log V relationship appears to be non linear, but this is solely due to a "hanging cloud" of very small plants that had not completed reproduction, a common phenomenon described in a recent review of R–V relationships (Weiner et al., 2009). We addressed this by setting a cut-off size of −0.75 for log (grain mass), which corresponds to 62 mg, for inclusion in the allometric analyses. The excluded points were from the smallest individuals at the highest densities. With this threshold, there was no evidence allometric relationships were non-linear (Fig. 1): second-degree polynomial terms (i.e. x^2) were not significant for any variety. Log (vegetative biomass) alone accounted for 92% of the variation in log(grain mass), without distinguishing among the varieties or densities.
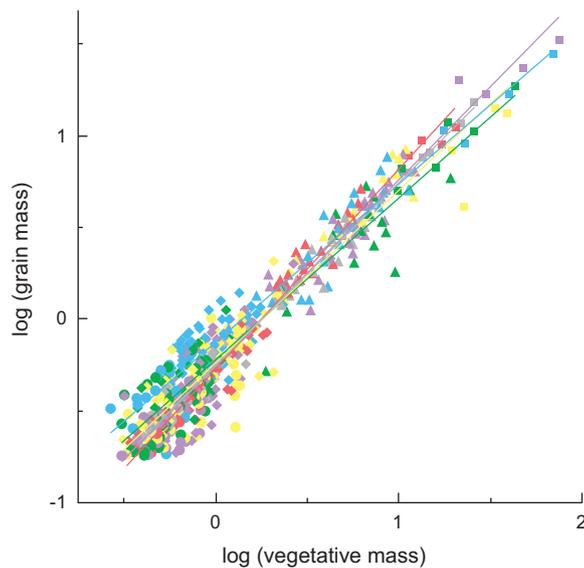
Fig. 1. Allometric relationship between grain mass and vegetative mass (all non-grain biomass) for 6 varieties of wheat (*Triticum*) grown at 4 densities. The colors represent the varieties (gray: MO4; red: MO1; yellow: DM22; green: DM31; blue: HST; violet: L8139) and the symbols represent the density (■: 10; ▲: 100; □: 1000; ●: 3000 m^{-2}). To remove very small individuals that had not completed reproduction, individuals with grain mass < 62 mg were excluded from the analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Allometric relationship between log(grain mass) and log(non-grain mass) for individuals of 6 varieties of *Triticum* (wheat) grown at 4 densities. Individuals with grain mass < 0.062 g were not included in the analysis (see text).

Variety	N	Slope ± S.E.	Intercept	R^2
MO1	72	1.088 ± 0.021	−0.279	0.984
MO4	83	0.997 ± 0.014	−0.260	0.916
DM31	92	0.883 ± 0.028	−0.229	0.917
DM22	101	0.942 ± 0.032	−0.246	0.896
HST	97	0.863 ± 0.022	−0.133	0.920
L8139	107	1.016 ± 0.026	−0.263	0.932

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fcr.2012.12.011.

The varieties varied in their allometric slopes (Table 1). The two diploid varieties had steeper allometric slopes than the two tetraploid varieties, while the slopes of the two modern varieties were significantly different from each other. Two varieties, DM31 and HST, had allometric slopes that were significantly ($P < 0.001$) less than 1 (Table 1). One variety (MO1) had a slope that was significantly ($P < 0.001$) greater than 1. The other 3 varieties had slopes that were not significantly different from 1.

In a complete analysis on log R, the dominant factor was log V, but variety, density and a variety × log V interaction were all highly significant (Table 2).

Table 2

Mixed linear model analysis of log(grain mass) on variety, log density, log(vegetative mass). Individuals with grain mass < 0.062 g were not included in the analysis (see text). Interactions with $P > 0.1$ are removed from the analysis. Adjusted r^2 for the model is 0.94.

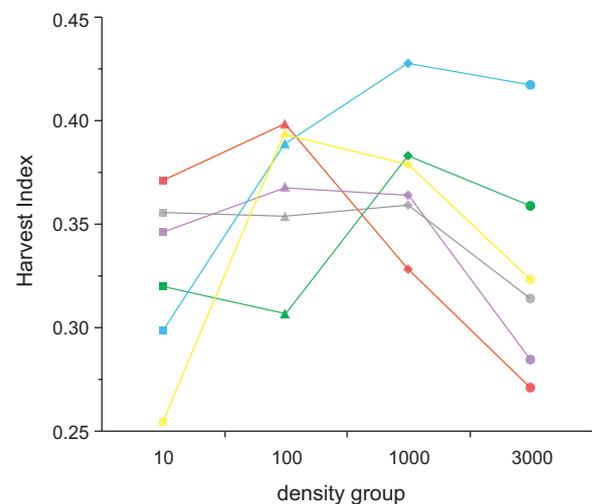| Source	SS	df	F	P
Variety	0.990	5	14.0	<.0001
Log density	0.366	1	25.9	<.0001
Log (vegetative mass)	24.789	1	1750.7	<.0001
Variety × log (vegetative mass)	0.656	5	9.3	<.0001

Fig. 2. Harvest Index of the varieties (gray: MO4; red: MO1; yellow: DM22; green: DM31; blue: HST; violet: L8139) at the four densities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In a traditional analysis of the Harvest Index at the population level as a function of the variety and density treatment (Fig. 2) showed no clear pattern except that HI was lower at the highest density than the next highest for all varieties. In a linear model analysis, neither variety nor density had significant effects on population HI (variety: SS = 0.006, df = 5, P = 0.70; density treatment: SS = 0.012, df = 5, P = 0.14).

4. Discussion

4.1. Size variation and allometry

Most of the variation in individual yield (grain mass) was due to plant size. Within a population, plant size is usually the best predictor of reproductive output. For a given genotype, obtaining high grain yield in the field is primarily about achieving high biomass production.

Although vegetative biomass was by far the most important factor in accounting for variation in grain biomass, variety, sowing density, and a vegetative biomass x variety interaction also had highly significant effects. The significant biomass × variety interaction means that the varieties differed in their allometric slopes. Some varieties produced more yield than others when plants were small, but less than the others when plants were large (Fig. 1). HI decreased with size in two varieties, increased in size in one variety and was not size-dependent in the remaining three varieties.

The significant effect of density (Table 2) means that there are effects of density in addition to those mediated by size. A relatively large individual at high density that is the same size as a relatively small individual at low density will produce slightly less yield. While size accounts for most of the variation among as well as within densities, individuals pay a cost of competition in addition to that due to size. In short, size is by far the most important determinant of individual yield for a genotype, but it is not the only determinant.

4.2. Size dependency of the Harvest Index

When the relationship between size and reproductive output is allometric in the broad sense (i.e. non-isometric) the HI of individuals will change with size. In such cases it is not meaningful to talk about the HI of a genotype. Plant breeders would benefit

from an allometric perspective on the relationship between total biomass and harvestable yield, rather than relying on HI or other ratios (Jasienski and Bazzaz, 1999; Weiner, 2004).

Research on Harvest Index has not taken the size-dependence of reproductive output into consideration. For example, one study found that modern genotypes had higher HI under high water conditions, but lower HI under in very dry conditions (Li et al., 2002). In the allometric view, the difference reflects the difference in the range of individual size under the two water conditions. HI is primarily the result of biomass production and allometric growth; it is not genetically determined in a direct way. It is the $R–V$ relationship that appears to be genetically determined and relatively fixed (Weiner et al., 2009). A traditional analysis of our data, looking at the HI of the population across the densities for each variety showed that at low densities (large plant size) the HI decreases in the order MO1 > MO4 ∼ L8139 > DM31 > HST > DM22 and that at high densities (small plant size) the HI decreases in the order HST > DM31 > DM22 ∼ MO4 > L8139 ∼ MO1 (Fig. 2). Thus, at the extremes of density (and therefore plant size) the order of HI is roughly similar to the one predicted from the slopes of the allometric relationships. For the genotypes with allometric slopes different from 1, the pattern of HI is consistent with the allometric regressions, i.e. the HI of MO1 decreases with increasing density, while that of DM31 and HST does the opposite. The results show that we should not interpret HI without taking consideration of the effects of size. We argue that the allometric analysis of individuals can be more informative and interpretable than an analysis HI alone.

Grasses adjust growth and tillering according to the space and resources available while they are growing. If the density is very low, the plant population cannot intercept all available resources, so biomass production will be lower than its potential. Also, when grown at low density, individuals of a tillering grass like wheat will develop many tillers, some of which will be initiated late in the growing season and not have sufficient developmental time to produce spikes, flowers and mature seeds. Thus, reproductive output will be lower than the biomass production could support. Over a wide range of densities, biomass production per unit area is the same, a phenomenon called "Constant Final Yield" (Shinozaki and Kira, 1956; Willey and Heath, 1969; Weiner and Freckleton, 2010). Plant parts such as reproductive structures do not usually show Constant Final Yield. Reproductive output per unit area decreases at very high densities, because many plants will be below the minimum size necessary for flower and fruit production.

Domestication of cereals over the past 10 000 years was performed by observation and direct selection of above-ground organs. Our results support the conclusion that the improvement in wheat yields was the consequence of the interaction of several factors, especially improved agronomic practices, which increased biomass production, followed by an increase in the Harvest Index (Van Dobben, 1962; Austin et al., 1980; Slafer et al., 1990; Reynolds et al., 1999; Brancourt-Hulmel et al., 2003; Royo et al., 2007; Khodarahmi et al., 2010). Breeders are interested in response per unit area, not in response per individual plant, and they often select for high Harvest Index per unit area under very favorable conditions in which plants are very large and individual variation smaller than under normal field conditions. Selecting for higher yield will often involve selecting for higher plant size, but due to reproductive allometry, increases in plant size will not always entail similar gains in yield. For example, breeding for higher reproductive potential, through increasing flower numbers per inflorescence, has not been matched by absolute increases in nutrient levels reaching these additional reproductive units (Ruan et al., 2012). Our results suggest that reproductive allometry should be explicitly incorporated into breeding programs and emphasis given to slopes and/or intercepts of the allometric relationship between vegetative and reproductive mass, rather than to yield at the population level alone.

Even though the farmer is interested in production per unit area, this is ultimately a result of the behavior of individual plants, so it is important to understand and analyze the behavior of individuals, not just the population as a whole (the mean individual), when there is variation among individuals, as in most agricultural fields. Our results suggest that smaller size and greater variation under field conditions, especially where inputs are limited, may mean that varieties selected for high HI under very favorable conditions may often be sub-optimal in the field.

5. Conclusions

Cereal breeders should focus on reproductive allometry of individuals when interpreting the Harvest Index, and select for allometric patterns that are most advantageous in a given agronomic context, especially when there is large variation in productivity among individuals, locations or years.

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