國立臺灣大學生命科學院生態學與演化生物學研究所

碩士論文

Institute of Ecology and Evolutionary Biology

College of Life Science

National Taiwan University

Master Thesis

從阿拉伯芥不同族群之性狀差異探討雜草演化

Investigating the phenotypic and genetic basis of weedy plant evolution in *Arabidopsis thaliana*

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中華民國 107 年 7 月

July 2018

doi:10.6342/NTU201801769

誌謝

這個研究首先要感謝中研院分生所蔡宜芳老師實驗室的協助，實驗中幾乎所有的阿拉伯芥品系都是從他們那裡取得種子的。且在實驗室的生長室尚未建設完成時，蔡老師實驗室提供了非常多的人力物力協助我將大量的阿拉伯芥種植在那邊，每次過去看到他們的生長室內幾乎一半以上都塞滿我的盆栽就會覺得有點不好意思使用了他們那麼多的資源。另外也謝謝植科所謝旭亮老師實驗室提供光照實驗以及下胚軸長度測量的技術操作指導。

最重要的還是要特別感謝實驗室的每個成員和李承叡老師，能參與實驗室的創立初期真的是很寶貴的經驗。原本升碩二時剛聽到有新生要進來是感到非常緊張的，因為自己其實不太知道實驗室的成員間互動應該要是什麼樣子或是實驗上要怎麼協調。雖然很享受第一年學生空間只有一個人，很空曠的環境，但還是比較喜歡之後實驗室成員增加，有人可以討論和給予我很多實驗上的建議，大幅增加學習以及實驗效率。特別感謝許哲維協助 F_{ST} 的分析，以及丁芯奕協助測量所有植株的性狀。另外也感謝李老師的指導，這段期間學習到了很多之前完全沒想過的有趣事物。前不久跟好友吃飯時聊到自己的實驗，跟對方解釋 F_{ST}-Q_{ST} 比較，吃完飯後才發現聊到聲音都沙啞了。這對我來說是小學到大學的過程中從來沒有過的經驗，升研究所前從沒有想過自己可以這麼沒壓力的分享學術上的事物，我很喜歡念研究所的這段日子，謝謝這段期間給我幫助的人。最後想額外感謝我萌萌的實驗材料阿拉伯芥，這個研究真的讓我對這個物種完全改觀，期待有一天能和他在野外相見。

羅澄玉 謹誌於

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中華民國一百零七年七月

doi:10.6342/NTU201801769

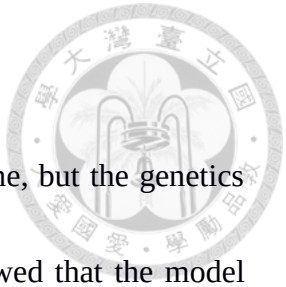
摘要

雜草所具的特徵過去已有許多研究探討過，但關於雜草演化的遺傳機制尚不明瞭。模式物種阿拉伯芥（*Arabidopsis thaliana*）經先前的研究發現現今分布於南歐半島、非洲的「子遺族群」過去曾分布於整個歐亞大陸，但雜草般的「非子遺族群」取代了子遺族群並散佈至世界各地。儘管對於阿拉伯芥的性狀已有非常多的研究，但過去研究所使用的品系幾乎都來自於非子遺族群，鮮少有研究探討不同族群間的差異。比較子遺和非子遺族群間的性狀差異可使我們能更進一步了解雜草的演化。本研究選了 52 個來自野外的阿拉伯芥自交品系，其中 31 個來自非子遺族群，21 個來自子遺族群，將這些品系種植在相同的環境條件下以比較兩個族群由遺傳造成的性狀差異。研究中主要測量了兩個與雜草有關的性狀：幼苗避陰反應及種子產量。幼苗避陰實驗將阿拉伯芥種子種植在兩個不同光照環境下，比較不同族群在不同光照處理下幼苗下胚軸長度的差異。結果顯示幼苗下胚軸長度在不同處理下有顯著差異，但不同族群間幼苗下胚軸長度以及在不同處理下的可塑性並沒有顯著差異。種子產量的實驗中比較了兩個族群的果實及種子相關性狀。結果顯示不同族群的種子大小及種子產量有顯著差異，子遺族群的種子大小平均比非子遺族群的大了 33%，但非子遺族群平均能產出將近子遺族群兩倍的總種子數。透過 F_{ST}- Q_{ST} 的比較，我們認為這些存在於子遺族群和非子遺族群間的性狀差異很可能是天擇推動造成的。種子產量的差異使阿拉伯芥非子遺族群很可能有著比子遺族群高的適存度，使其能取代子遺族群並廣泛散佈。此研究結果未來可進一步利用數量表徵基因座定位法及全基因組關聯分析找出控制阿拉伯芥非子遺族群成為雜草的候選基因，以了解雜草演化的遺傳機制。

關鍵字：

阿拉伯芥、雜草、自然變異、避蔭反應、種子產量、F_{ST}-Q_{ST}比較

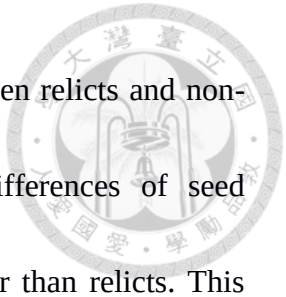
doi:10.6342/NTU201801769



Abstract

The characteristics of weedy plants have been studied for a long time, but the genetics of weedy plant evolution remains unclear. Previous researches showed that the model plant *Arabidopsis thaliana* consists of multiple genetic groups. The "relict" populations currently found in Southern Europe and Africa once occupied most of Eurasia, but the weedy "non-relict" population rapidly replaced the relict populations and spread worldwide. While many traits of *A. thaliana* have been much studied, most laboratory strains were from non-relict populations, and few studies have investigated the differences between relicts and non-relicts. Comparing the phenotypic variances between these two groups provides a way to study the genetics of weedy plant evolution. Fifty-two accessions of *A. thaliana* were used in this study, including 31 non-relict and 21 relict accessions. All accessions were grown in controlled conditions to compare their phenotypic differences. Two types of traits associated with weediness were measured: shade avoidance response and seed productivity. For shade avoidance response, we treated the seedlings with two different light conditions. The hypocotyl length of seedlings showed significant differences between treatments but not between populations. For seed- and fruit-related traits, we observed significant differences for seed size and productivity: Seeds from relicts were about 33% bigger than non-relicts on average, but non-relicts yielded almost twice as many seeds as relicts. According to

doi:10.6342/NTU201801769



the results of F_{ST}-Q_{ST} comparison, the phenotypic differences between relicts and non-relicts might be caused by natural selection. Owing to the differences of seed productivity, non-relicts might have higher fitness and spread faster than relicts. This research lay a foundation for further quantitative trait loci mapping and genome-wide association study to find candidate genes controlling weedy phenotypes.

Keyword:

Arabidopsis thaliana, weed, natural variation, shade avoidance response, seed productivity, F_{ST}-Q_{ST} comparison

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Introduction

Arabidopsis thaliana is a Brassicaceae species native to Eurasia and Africa. It's weedy characteristics like short life cycle and wide distribution make it a model plant not only in molecular biology but also in ecology and evolution studies [1, 2]. A recent study performed genome re-sequencing of 1135 worldwide natural inbred lines (call ecotypes or accessions) of *A. thaliana* and the results show that this species consists of multiple genetic groups [3]. Since those genetic groups diverged during the last ice age, the researchers call the populations currently found in Southern Europe and Africa "relict" and the worldwide population "non-relict". Follow-up investigations showed that the relict populations once occupied Eurasia after the last ice age, but the population from Balkans not only expanded northwards but also expanded along the east-west axis of Eurasia and quickly replaced other populations [4]. The reasons why non-relicts could replace relicts quickly remain obscure. Although *A. thaliana* is a common model species, most studies used accessions from non-relict population for experiments, and few studies have investigated the difference between relicts and non-relicts. Owing to its wide distribution in agricultural and artificial areas, non-relicts have been considered as human commensal weeds [3]. The characteristics of weedy plants have been studied for a long time, such as short life cycle, long-distance dispersal, high

doi:10.6342/NTU201801769

seed output [5-7]. To investigate the differences between relicts and non-relicts and answer the question how non-relicts became weedy, we focus on two types of traits that associated with weediness: plasticity of hypocotyl length (shade avoidance response) and seed productivity.

Plasticity refers to the ability of an organism to change its behavior, morphology or physiology in response to different environments without genetic changes. High plasticity has been viewed as one of the ideal characteristics of weeds because it allows the weedy species to adapt to areas with large environmental changes [8-10]. One of the classic plastic trait of plants is shade avoidance response. The ratio of red to far-red light (R:FR) is reduced under canopy shade because plant pigments absorb red light while far-red light transmits through the canopy relatively unimpeded [11]. Plants can sense the change of light condition by phytochromes resulting in a suite of plastic responses such as elongated hypocotyls, internodes, and acceleration of flowering [12-14]. A previous study has compared the hypocotyl length of Cvi (a relict from Cape Verde Islands) and Ler (a non-relict from Germany) accessions of *A. thaliana* under different light condition [15]. The results showed that hypocotyl length of Cvi is generally longer but has weaker response than Ler because Cvi's variation of hypocotyl length under different treatments is lower than Ler. We could only know the differences between two

doi:10.6342/NTU201801769

accessions from previous study, and it remains unknown whether this is a general difference between relicts and non-relicts since the relict genetic groups were only discovered in 2016. We expect that non-relicts might have shorter hypocotyl length but higher plasticity under different environments. In our study we treated the seedlings of both relict and non-relict with two different light conditions then compared the hypocotyl length between treatments and populations to test our hypothesis.

High seed productivity is another important characteristic of weeds. Tradeoffs between number of seeds and seed size have long been studied. Weeds tend to produce smaller but more seeds, which make them disperse more easily [5, 16]. A previous study also compared differences of traits between Cvi and Ler. The results show that seeds of Cvi were much larger than Ler, while total seed yield of Ler is 40% higher than Cvi [17]. Another study collected 300 accessions from the Iberian Peninsula and measured their seed size [18]. We reanalyzed their data and found that seed size of relicts in Iberia was significantly larger than non-relicts. Based on the results of the above two studies, we expect the total seed number non-relicts can produce is higher than relicts because relicts have relatively large seeds and there might be tradeoffs between seed size and yield [17].

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In addition to identify phenotypic differences between relicts and non-relicts, we also need to distinguish whether the differentiations of those traits were caused by natural selection or genetic drift. F_{ST}-Q_{ST} comparisons provide a way to answer this question. F_{ST} describe the magnitude of genetic differentiation between populations for a genetic locus. Q_{ST} measures the amount of heritable trait variance among populations relative to the total trait variance, similar to F_{ST}. If populations are under divergent selection, F_{ST} of selected loci is expected to be higher than neutral loci [19, 20]. Q_{ST} of a neutral quantitative trait is generally equal to F_{ST} of neutral loci [21]. Therefore, we can use F_{ST} of neutral molecular markers as a null expectation for population differentiation caused by genetic drift [22, 23]. If Q_{ST} roughly equals F_{ST}, the divergence of trait between populations might be due to genetic drift alone. If Q_{ST} is higher than F_{ST}, the divergence of trait is likely to have been caused by divergent selection. In order to avoid false positive results, previous paper suggested that genome-wide F_{ST} distribution should be used to compare with Q_{ST} of each trait, not mean F_{ST} [24].

Here we test whether the above-mentioned weediness-related traits are significantly different between relicts and non-relicts. Fifty-two accessions including 21 relicts and 31 non-relicts were chosen and grown in common environments to standardize the influences of environment on phenotypes. After trait comparisons, we

doi:10.6342/NTU201801769

further perform $F_{ST} - Q_{ST}$ comparisons to test whether the phenotypic divergence was caused by natural selection or random genetic change. Although the characteristics of weedy plants have been studied for a long time, the genetics of weedy plant evolution remains obscure. Our aim is to find traits highly diverged between relicts and weedy non-relicts for further genetic mapping to identify candidate genes controlling those weedy phenotypes.

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Materials and Methods

Plant materials

Fifty-two accessions of *A. thaliana* were used in this study, including 31 non-relict and 21 relict accessions (Figure 1, Table 1). Non-relict samples were chosen from the accessions collected in Eurasia and a laboratory strain Col-0 from North America. The non-relict accessions we chose were known to have little introgressions from relict populations [4], in order to avoid phenotypic variance caused by gene flow with relicts. Relict samples included the accessions from known genetic groups in Cape Verde, Iberia, Sicily, Lebanon and Tanzania. Seeds of all accessions obtained from the stock center were first grown under 22℃, long day light (16 hours light: 8 hours darkness) for one generation to bulk up the seeds and eliminate possible maternal effects. Next-generation seeds were used for following experiments. Since *A. thaliana* is a mostly self-fertilizing plant [25, 26] and these accessions have been inbred in the lab for several generations, all seeds in the same accession are clones of each other and can be viewed as replicates in randomized experiments.

Hypocotyl length measurements

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Seeds were sterilized in 70% ethanol with 0.01% Triton X-100 for 10-min, followed by a 10-min wash with 95% ethanol, and then resuspended in 1 ml sterile water [15]. After sterilized, seeds were plated on 0.7% agar gel containing 1x Murashige and Skoog medium (Duchefa, Product code: M0222) with 0.3% sucrose and 0.05% MES (2-(N-Morpholino)ethanesulfonic acid, CAS Number: 145224-94-8) in petri dish (diameter: 9cm, height: 2cm). The spacing between each seed was at least 0.5 cm to avoid the seedlings from interfering with each other. Each plate contained four accessions and at least 20 seeds of each accession were placed. Our experimental procedure follows a previous study investigating natural genetic variation of hypocotyl response in *A. thaliana* (but without relicts) [27]. Plates were kept in the dark at 4℃ for one week and then moved to LED growth chambers with long day light condition (16 hours light: 8 hours darkness) at 22℃. Light condition was set to 34 $\mu E/m^2/s$ red light and 7 $\mu E/m^2/s$ blue light to induce germination. After 24 hours, far-red light was added to bring the ratio of R:FR to 2 (Figure 2A). After an additional 48 hours, the setting of R:FR ratio in shade simulated experiments was lower to 0.5 (Figure 2B) while the R:FR ratio in sun simulated experiments were kept at 2 for four days. Spectrometer (HiPoint, HR350) was used to measure the light condition inside the LED growth chamber in

doi:10.6342/NTU201801769

order to adjust the settings to the conditions we need. The entire experiment replicated two times for each treatment.

In order to avoid hypocotyl length variation due to the adhesion on agar gel, the seedlings lying on the agar gel surface were excluded from further analysis. A scanner (MICROTEK, ArtixScan F2) was used to scan all seedlings to image files with the resolution at 2000 ppi. Hypocotyl length of each seedlings was measured using ImageJ [28]. Since we scanned the seedlings with ruler, we can transform the unit from pixel to millimeter by “Set Scale” function in ImageJ. The shade avoidance response of each accessions was calculated by hypocotyl length under R:FR = 0.5 minus hypocotyl length under R:FR = 2.

Measurements of fruit and seed-related traits

Un-sterilized seeds of all 52 accessions were suspended in 1 ml sterile water and kept in the dark at 4℃ to break dormancy and synchronize germination. After one week, multiple seeds from each accession were sown in pots containing sterile soil (BVB substrate : KING ROOT plant medium = 2:3) and moved to growth chamber with long day light (16 hours light: 8 hours darkness) at 22℃. Every 20 pots are placed in a rectangular plastic basin (length x width x height = 45 x 35 x 11 cm) and watered twice

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a week. When watering, 700 ml H_2O was poured into the plastic basin. After an additional week, extra seedlings were removed so that there were only one seedlings per pot. The randomized block experiment consists of four blocks, each block contains one individual plant from each accession. The whole experiment therefore has 52 x 4 = 208 plants in total. Two weeks after this thinning, the accessions whose flowering time longer than 3 weeks were moved into 4℃ refrigerator with fluorescent lamps for five weeks for cold vernalization, while others were kept in the growth chamber (Table 1). After the plant flowered and withered, the following traits were measured for further analyses. The accessions couldn't grow to bloom and bearing fruits (can't bloom after vernalization or dead before flowering) were excluded from further measurements. The accessions used for final analysis were recorded in Table1.

Number of fruits (FN) of each plant were recorded and undeveloped fruits were excluded from the measurement. Following the previous study [17], for each plant we harvested one fruit from the main inflorescence at a position 6 to 10 from the lowest fruit measured the following traits: seed number per fruit (SNF), number of unfertilized ovules per fruit (UOF), ovules number per fruit (ONF), fertilized percentage (FP) and fruit length (FL). SNF and UOF were counted under a stereomicroscope. ONF was obtained as SNF + UOF, and FP is (SNF / ONF) × 100%. Seed number per millimeter

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(SNM) and ovule number per millimeter (ONM) of each plant were estimated separately respectively as SNF/FL and ONF/FL. Total seed number (SN) and ovule number (ON) of each plant were estimated respectively as SNF × FN and ONF × FN. For seed size measurement, 10 seeds from each plant were placed on slides and scanned by a scanner (MICROTEK, ARTIXSCAN F2) with the resolution at 2000 ppi [29]. All image files of the seeds were analyzed using the particle analysis function of ImageJ and the following measurements were recorded: area (seed size, SS), maximum Feret diameter (seed length, SL), and minimum Feret diameter (seed width, SWi). Because the related traits were measured from image files, we used pixel as the unit of seed size and length.

Statistical analysis

Univariate mixed-model analysis of variance (ANOVA) implemented in JMP 13 (SAS, Cary, NC, USA) was used for all analyses. For fruit- and seed-related traits, "accession" was used as a random effect nested within the "relict" fixed effect. The binary "relict" effect denotes whether an accession belongs to the relict or the non-relict group and therefore has one degree of freedom. For the experiment of shade avoidance

doi:10.6342/NTU201801769

response, we added a “treatment” (two light conditions, R:FR = 2 and R:FR = 0.5) fixed effect as well as the fixed interaction effect between “relict” and “treatment”.

To investigate the relationship between different trait, least square mean of each trait from each accession was used to calculated the correlations and the correlation matrices were estimated by package “PerformanceAnalytics” in R.

$F_{ST} - Q_{ST}$ comparisons

Single-nucleotide polymorphism (SNP) data from 1001 Genome was used to estimate F_{ST} between 21 relicts and 32 non-relicts. Twenty thousand SNPs were randomly selected from whole genome SNPs and package HIERFSTAT in R was used to calculate the F_{ST} of each SNP.

In this study we used “relict” and accessions nested within “relict” both as random effects to estimate their variance components with JMP 13 (SAS, Cary, NC, USA). Q_{ST} of each trait was calculated as

$$V_{relict} / (V_{relict} + V_{accession}),$$

where V_{relict} and $V_{accession}$ represent the variance components of the relict and accession term respectively. The within-accessions variance component is usually multiplied by

doi:10.6342/NTU201801769

two in typical Q_{ST} formula. Since *A. thaliana* is a highly selfing species, it can be modelled as haploid in our calculations [24].

doi:10.6342/NTU201801769

Results

Hypocotyl length

We treated the seedlings with two R:FR ratio regimes to compare their response to different light conditions. As expected, the hypocotyl length of seedlings showed significant differences between treatments in both relict and non-relict populations (Figure 3, Table2). Seedlings grown in low R:FR condition (R:FR = 0.5) were about 33% taller than high R:FR condition (R:FR = 2). However, there is no significant difference (P = 0.2719) between the hypocotyl length of relicts (4.39 ± 0.33 mm) and non-relicts (3.93 ± 0.24 mm)(Table 2). The relict by treatment interaction effect is not significant either (p = 0.2332, Table 2), as shown by the parallel reaction norms of relicts and non-relicts across treatments (Figure 3).

Previous studies have found significant correlations in different hypocotyl-related traits and latitude [27], so we also perform same analysis to check whether similar pattern can be found in our results. Overall, the hypocotyl length under two treatments were highly positively correlated. It also showed significant positive correlation between height in low R:FR and response to low R:FR. Relationship of the high R:FR and response phenotypes show negative correlation but not significant (Figure 4A). Relationships between phenotypes in non-relict population also showed similar pattern,

doi:10.6342/NTU201801769

while there was no significant correlation between the low R:FR and response phenotypes in relict population. Instead, responses of hypocotyl length and high R:FR phenotypes showed significant negative correlation in relict population (Figure 4B, C). The hypocotyl length of both populations showed no significant correlations with latitude.

Seed productivity and size

To estimate the total seeds an accession can yield, first we measured the seed productivity in one fruit. Non-relicts had 32% more ovules and 39% more seeds per fruit than relicts (Table 3), while number of unfertilized ovules ($p = 0.9604$, Figure 5) and the percentage of ovules that have successfully grown into seeds ($p = 0.1726$, Figure 5) showed no significant differences between two populations. Since the fruit length showed no significant difference between relicts and non-relicts ($p = 0.5763$, Figure 5), non-relicts had higher density of seed and ovule in a fruit than relicts (Figure 5). After knowing how many seeds a fruit can produce, we estimated how many fruits an individual can have. Non-relicts also had more fruits than relicts on average, so the total seed productivity of non-relicts was almost twice as many as relicts on average. (Table 3 and Figure 6).

doi:10.6342/NTU201801769

Relicts and non-relicts differ in not only seed productivity but also seed size. In our experiment, the size of the seeds was represented by the projected area of the seeds. The results showed that seeds produced by relicts were 33% larger than non-relicts on average (Table 3). Both seed length and width of relicts were significantly longer than non-relicts. (Figure 7).

Since vernelization might affect the results of the traits, we also did statistical analysis excluding the accessions which flowered before vernelization. The results are consist: non-relicts have significantly higher seed productivity but smaller seeds than relicts. (Table 4)

In order to assess the relationship between seed size and productivity more thoroughly, we examined the correlations between different traits. When considering all accessions, size-related traits and seed productivity of a fruit were strongly negatively correlated (Figure 8A). This relationship was also found in non-relict population, while there was no significant correlation in relict population. As to the relationship between seed-size-related traits and fruit number, we observed a strong negative correlation when considering all accessions (Figure 8A). When the relict or non-relict group was analyzed separately, however, this strong relationship disappeared (Figure8B, C),

doi:10.6342/NTU201801769

denoting such correlation might be caused by the differences between relicts and non-relicts.

F_{ST}-Q_{ST} comparison

Our results showed that there were significant differences in seed productivity and seed size between relicts and non-relicts. We want to know whether those phenotypic divergences among populations were cause by natural selection or random genetic drift. Our F_{ST} distribution shows the upper tail 5% cut-off at 0.231 and 1% cut-off at 0.54 (Figure 9). Since Q_{ST} represent the divergence of quantitative traits among populations, those traits showed no significant difference between relict and non-relict have Q_{ST} values smaller than 5% cut-off of F_{ST}. Except fruit number, Q_{ST} values of the traits that were significantly different between relict and non-relict (Tale 3) are higher than 1% cut-off of F_{ST} (Figure 9). However, the Q_{ST} value of fruit number is still higher than the upper tail 5% cut-off of F_{ST}. Q_{ST} values analyzed from only those accessions treated with vernelization also show similar pattern. The Q_{ST} values of fruit number is higher than the upper tail 1% cut-off of F_{ST} in this result. The Q_{ST} values of seed width is lower than previous analysis but still higher than the upper tail 5% cut-off of F_{ST} (Table 4).

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The results suggest that the differences of seed productivity and seed size between relicts and non-relicts might be caused by divergent selection.

doi:10.6342/NTU201801769

Discussion

As a model species, *Arabidopsis thaliana* have long been used for genotypic and phenotypic study. With the progress of next-generation sequencing technology, we have a breakthrough discovery of the genetic history of *A. thaliana* in recent years. Reconstructing the history of *A. thaliana* provides us a larger scale to investigate the phenotypic changes. The aim of our study is to identify phenotypic differences between different genetic groups of *A. thaliana* which made one of the groups weedy. Unlike most studies using few accessions or accessions from only one genetic group, we selected accessions from all over Europe with roughly equal representation for relicts and non-relicts. In order to compare the relationship between traits and population structure more clearly, we grew those accessions in the same environmental conditions to make variation of traits mainly controlled by genetic differences. Results of our study suggest that some natural phenotypic variation found in few accessions are the generally differences between populations.

Previous researches suggested that the plasticity of shade avoidance response is an adaptive trait and loss of this plastic response might reduce the fitness of the plant under some environments [30, 31]. In addition, relicts are restricted to un-disturbed natural habitats which are probably more shaded, while non-relicts can live in human-disturbed

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environments [3]. Because previous research observed that shade avoidance response in species from shaded regions was lower than open regions [32], we expect that non-relicts might have shorter hypocotyl length but higher plasticity, which makes non-relict have higher fitness. The results of this experiment are not consistent with our hypothesis that relict accessions generally have longer hypocotyl length. There are also no significant interactions between the relict and the treatment term. This means the responses of relicts and non-relicts to different environments are similar, suggesting non-relicts do not have higher plasticity than relicts. Previous studies also found significant negative correlations between latitude and hypocotyl length [27, 33-35]. However, similar pattern cannot be found in our results.

Artificially disturbed regions are generally considered to be exposed to direct sunlight much easier than natural environments. However, relicts are mainly distributed in southern Europe which is a region with Mediterranean climates. Instead of dense forest, vegetation of Mediterranean climates is generally characterized by savannas, shrublands, and grasslands [36]. This might be the reason why the traits of hypocotyl length between relicts and non-relicts did not highly diverge. Although the experimental results of shade avoidance response did not meet our expectations, this is still the first investigation focus on comparison between different populations of *A. thaliana*. In

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addition, the responses to low R:FR of relicts and non-relicts were significantly correlated with length under high R:FR and low R:FR respectively. It suggests that the shade avoidance response of relict and non-relict might be attributed to different light conditions. Since we know little information about the microclimate conditions at the sites where these accessions were collected, further studies require field experiments. Local environmental variables need to be recorded to investigate local adaptation in shade avoidance response.

Results of seed productivity and size are in line with previous studies and our expectations. There is a general negative correlation between seed yield and size in *A. thaliana* (Figure 8). Non-relicts tend to produce smaller but more seeds than relicts. Previous studies have found that Cvi has longer fruits than Ler [17], however our study shows that such differences are not common between relict and non-relict (Figure 5, Table 3). Since there is no significant difference in fruit length, larger seeds of relicts make it hard to produce more seeds than non-relicts in limited space. Although some of the accessions in our experiment did not bloom and bear fruit successfully, those relict accessions that were not included in the results were all from the Iberian population (Table 1). Because the remaining accessions still include all relict populations found

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today (including the Cape Verde Islands, Sicily, Lebanon, and Tanzania), our study can still reflect the differences between the current relict and non-relict.

Fitness can be quantified sequentially from germination rate to flowering to fecundity, and the total seed number a plant can produce is one of the most important component affecting overall fitness [37]. In addition, smaller seeds have long been considered to have better ability to disperse [5]. Small but abundant seeds of non-relicts are probably one of the reasons why it can quickly disperse and replace relict. Although seed production of relict is far less than non-relict, larger seeds have been found in previous studies to have higher survival rates in certain environments, especially in hot and arid environments [38, 39]. For rosette drought tolerance, a recent study showed that the Q_{ST} value of the drought-survival index is not higher than F_{ST} between relicts and non-relicts [40]. Although relict have high drought-survival index, this difference was not caused by strong selection. This result suggests that drought tolerance might not be an important issue when investigating why non-relict become weedy, since non-relicts' being less drought tolerant is not a trait under strong directional selection.

Few studies have investigated the genetic and genomic architecture of weedy plant evolution. *A. thaliana* contains large genetic and phenotypic diversity and its distribution area covers a wide ecological and climatic diversity. Since natural

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population and weedy population coexist as within-species variation in *A. thaliana*, it provides a unique material for the studies of weedy plant evolution. Our study found some weed-related traits highly diverge between different population of *A. thaliana* which might cause by divergent selection. Further study can focus on those differences and perform genetic mapping to find candidate genes controlling weedy phenotypes. By genetic mapping, we can understand the evolution history of weeds more thoroughly.

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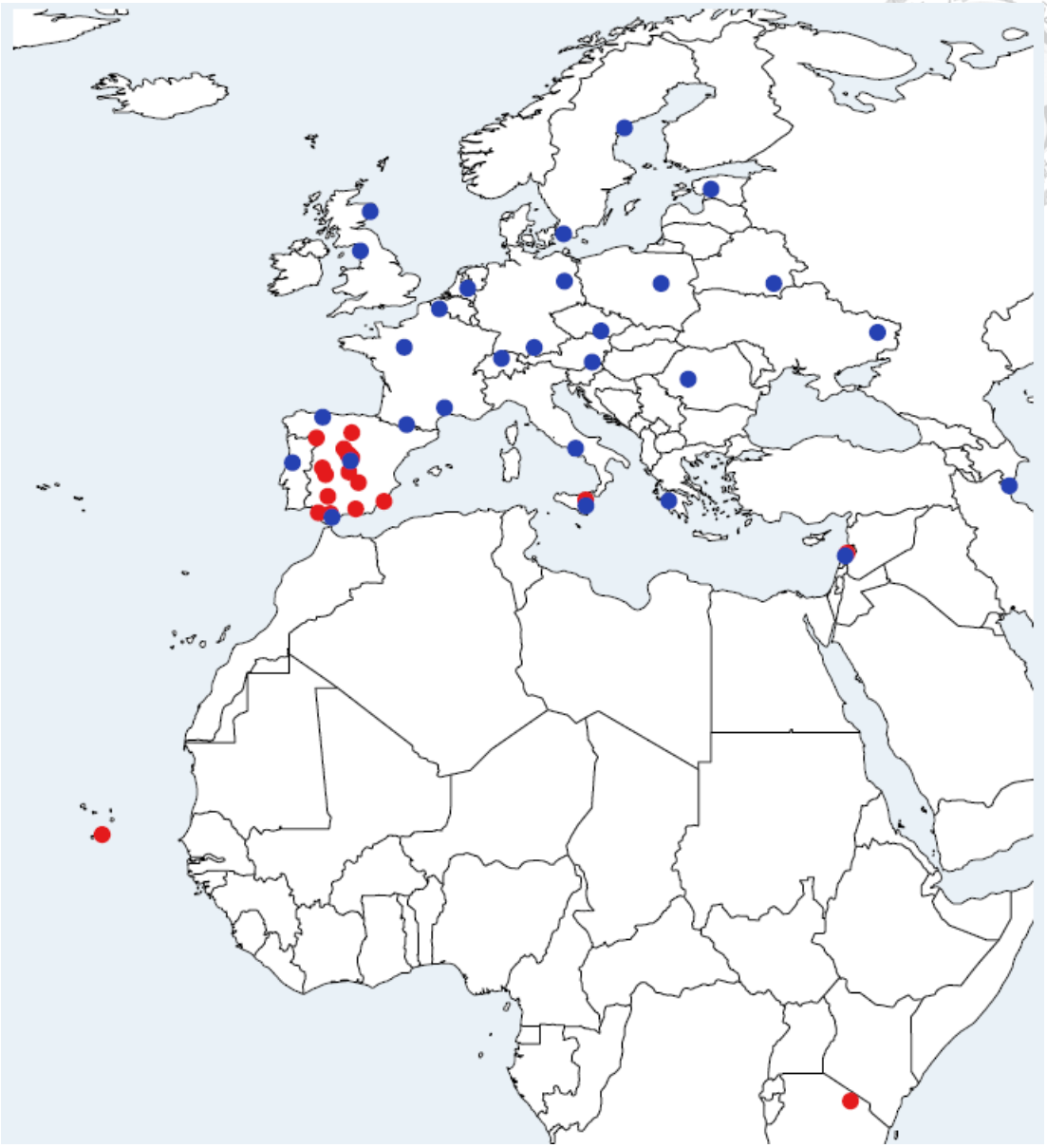

Figure 1. Origins of accessions used in this study.

Dots on the map show the collection location of accessions form Europe and Africa used in this study. Red and blue dots represent relicts and non-relicts respectively.

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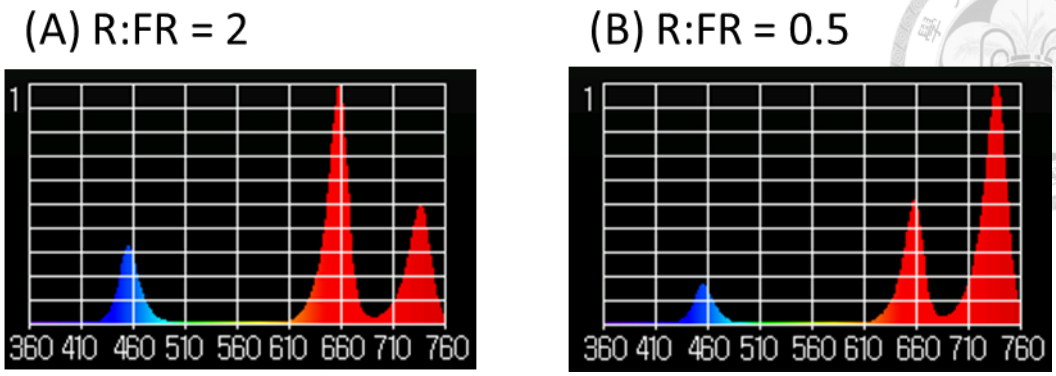

Figure 2. Spectrum of different light conditions in hypocotyl experiment.

The plots were generated by spectrometer (HiPoint, HR350). x-axis shows the wavelength (nm). The highest peak is viewed as 1 at y-axis.

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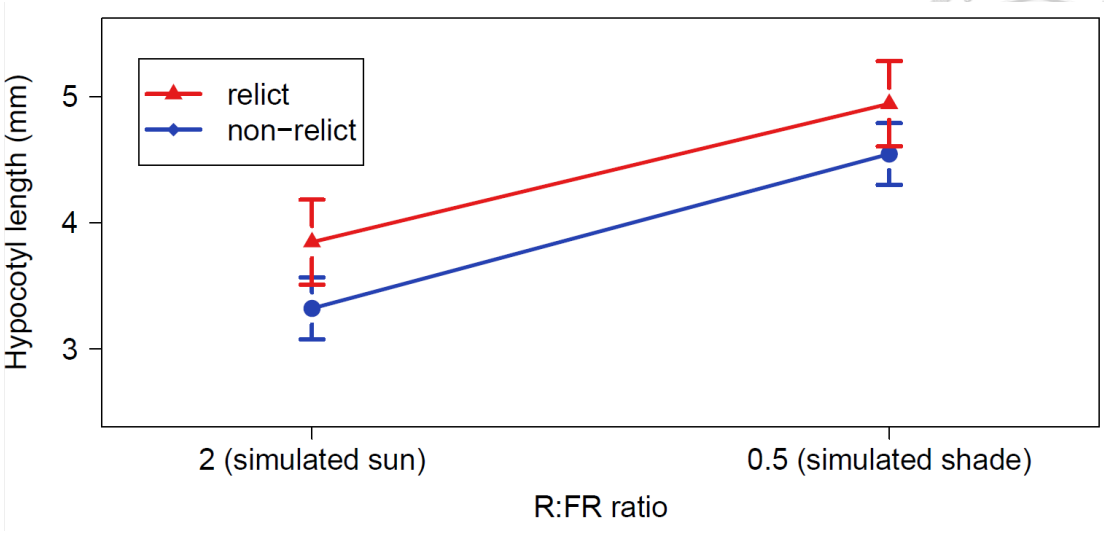
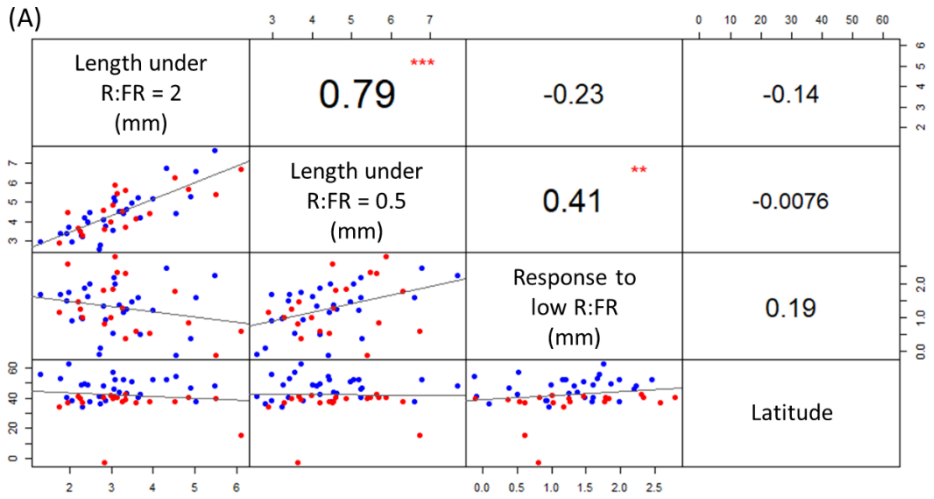
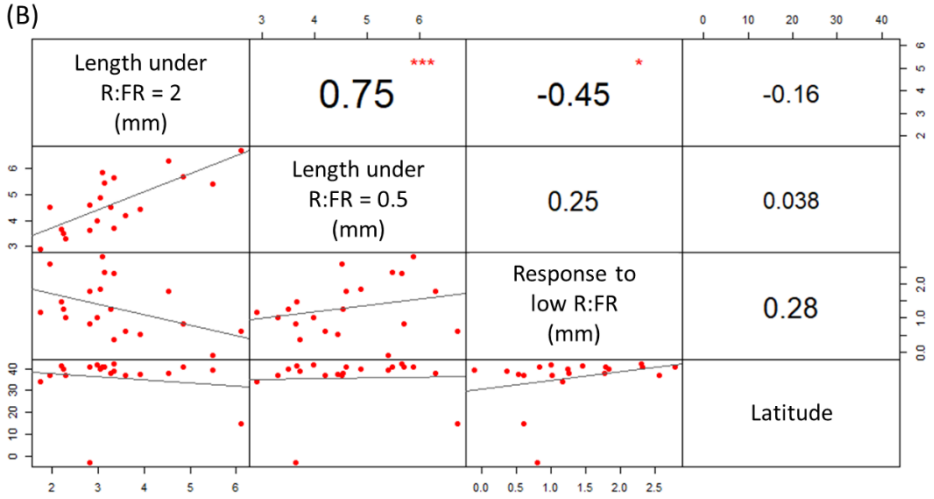


Figure 3. Hypocotyl length under different light conditions.

Shown are the hypocotyl length reaction norms of relicts and non-relicts. Red and blue dots show the mean hypocotyl length of two populations under two R:FR ratios and the bars on the dots show the standard error.

doi:10.6342/NTU201801769





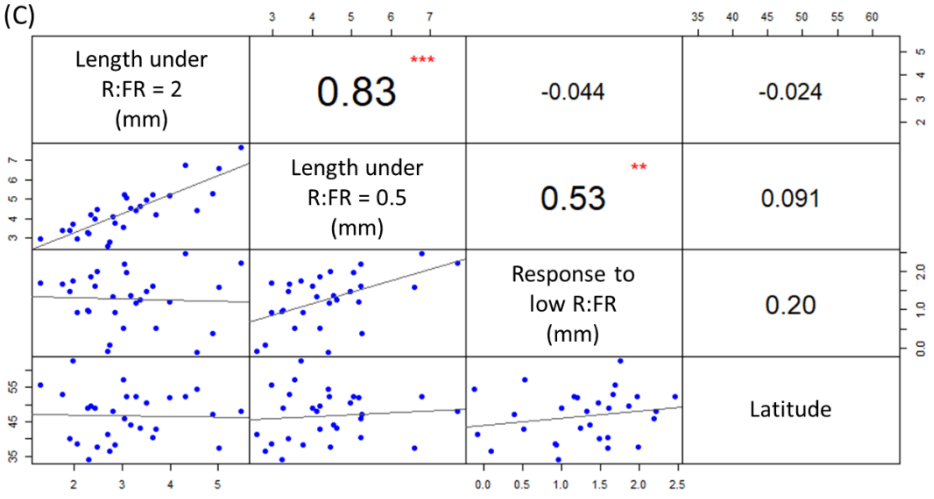


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Figure 4. Relationships between phenotypes of hypocotyl length and latitude.

(A), (B), (C) show the relationships within all accessions, relict and non-relict populations respectively. The lower triangle shows the scatter plots and regression line. Red and blue dots represent relicts and non-relicts respectively. Values in the upper triangle denote Pearson's correlation coefficient *r* and the corresponding significance (*: $0.01 < P <= 0.05$, **: $0.001 < P < 0.01$, ***: $P <= 0.001$).

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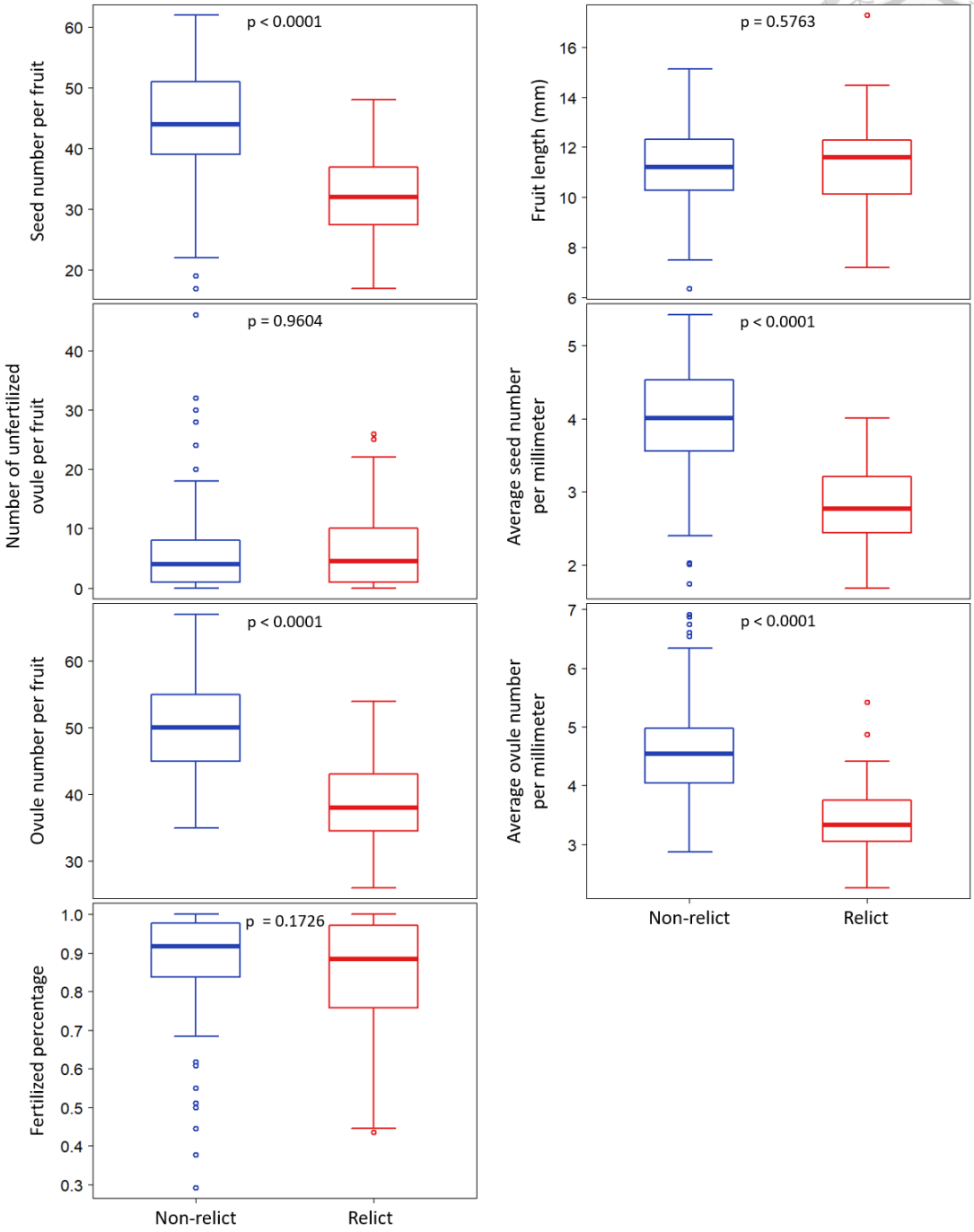

Figure 5. Traits of a fruit.

This figure shows boxplots of traits measured from a fruit of each plant. Relicts draw in red line and non-relicts draw in blue line.

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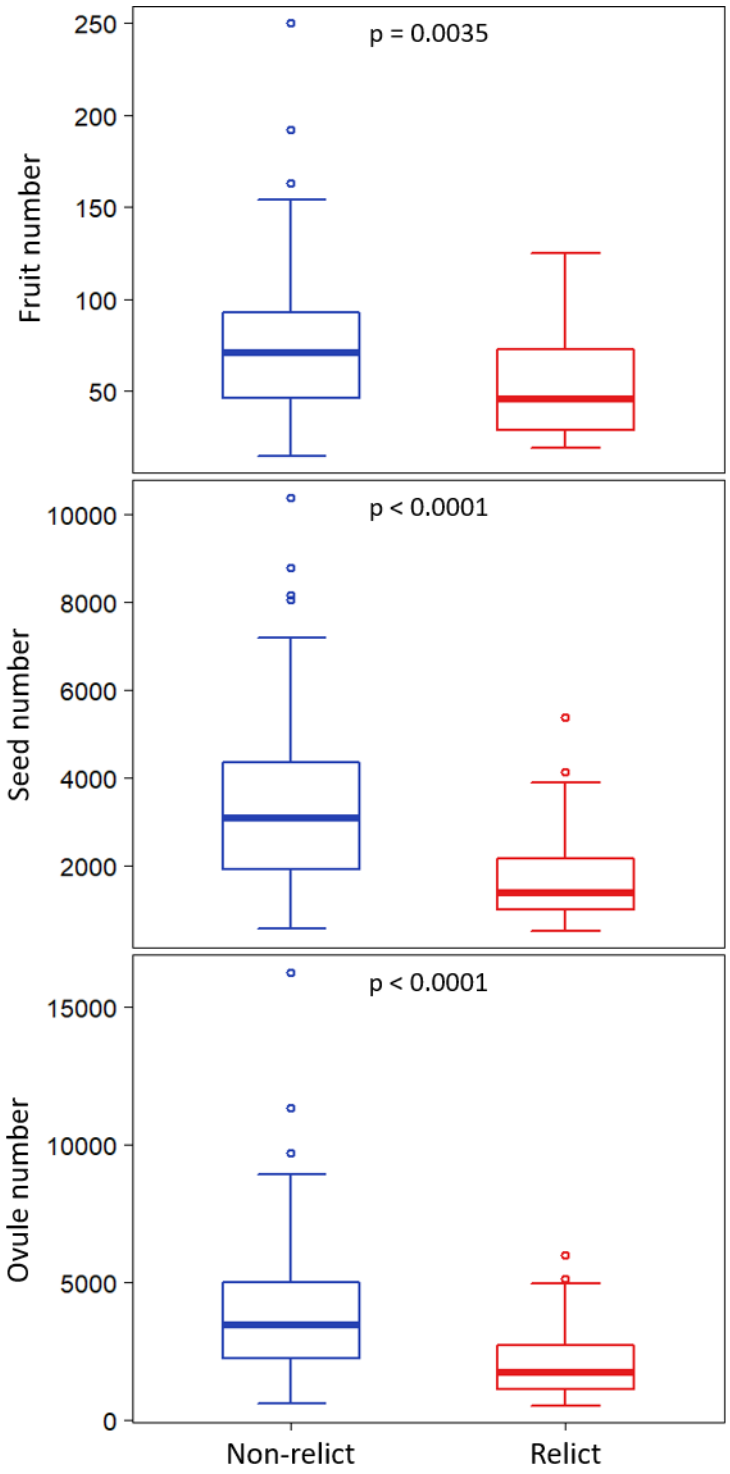

Figure 6. Traits of productivity.

This figure shows boxplots of traits related to seed productivity. Relicts draw in red line and non-relicts draw in blue line.

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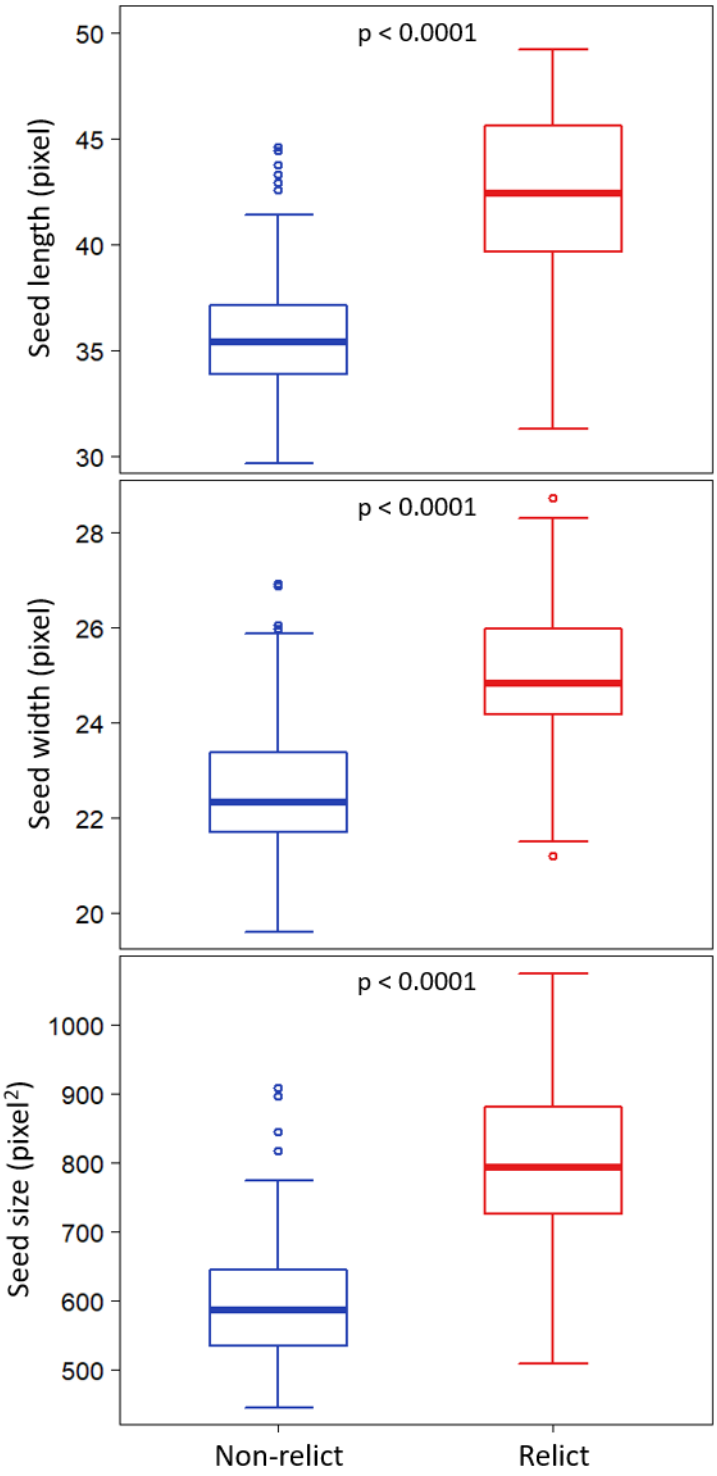

Figure 7. Traits of seed size.

This figure shows boxplots of traits related to seed size. Relicts draw in red line and non-relicts draw in blue line.

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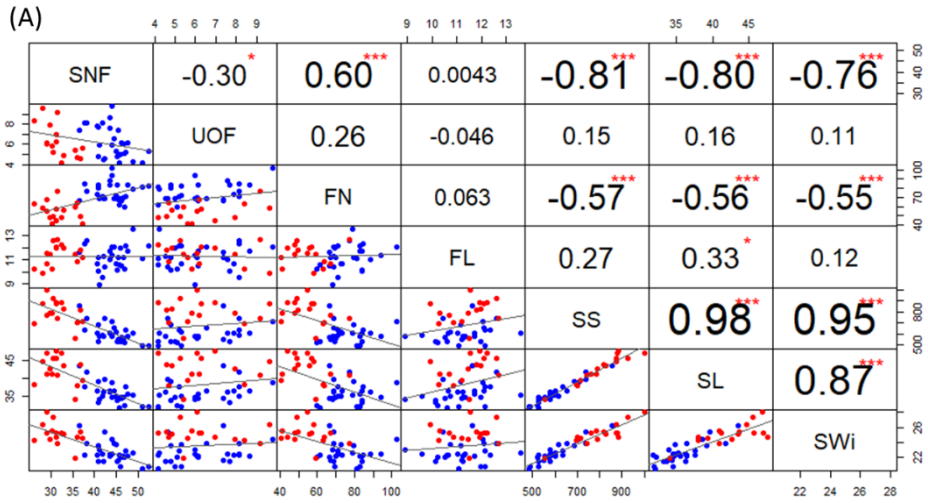

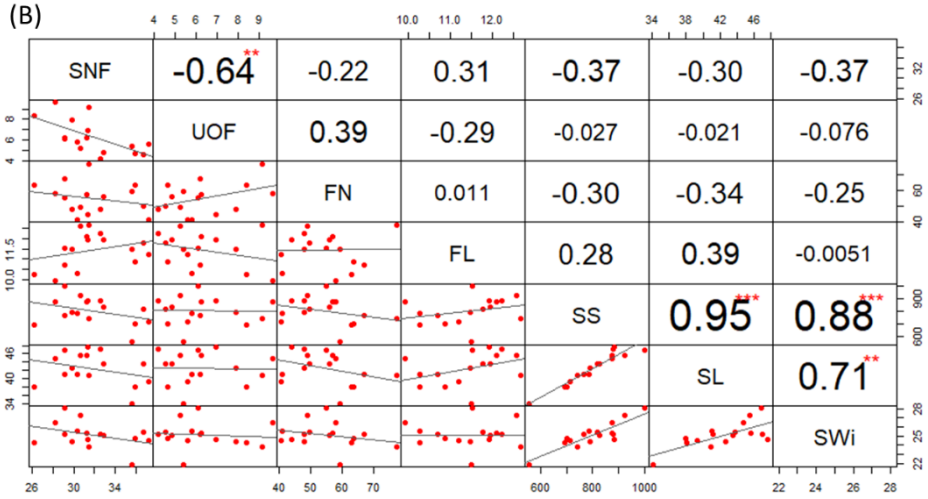

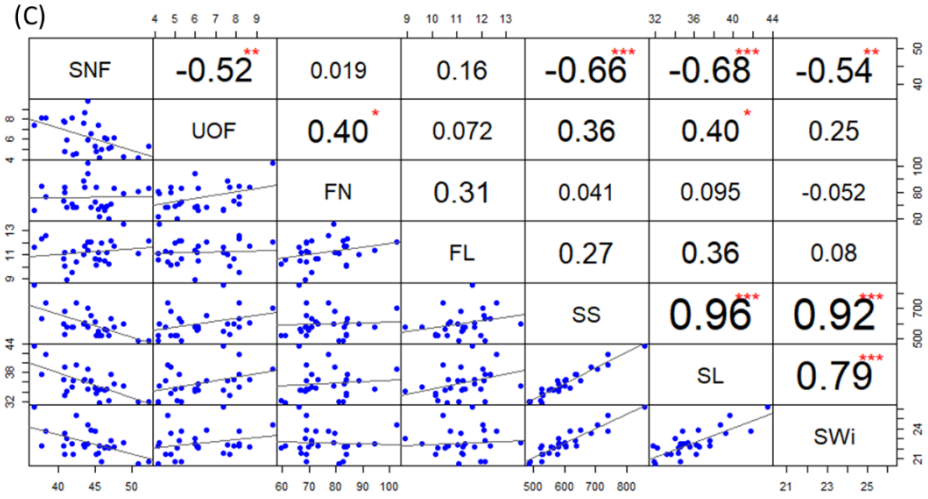

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Figure 8. Relationships of traits related to seed productivity and size.

(A), (B), (C) show the relationships within all accessions, relict and non-relict populations respectively. The lower triangle shows the scatter plots and regression line. Red and blue dots represent relicts and non-relicts respectively. Values in the upper triangle denote Pearson's correlation coefficient r and the corresponding significance (*: 0.01 < P <= 0.05, **: 0.001 < P < 0.01, ***: P <= 0.001). SNF: seed number per fruit, UOF: number of unfertilized ovules per fruit, FN: fruit number, FL: fruit length, SS: seed size, SL: seed length, SWi: seed width.

doi:10.6342/NTU201801769

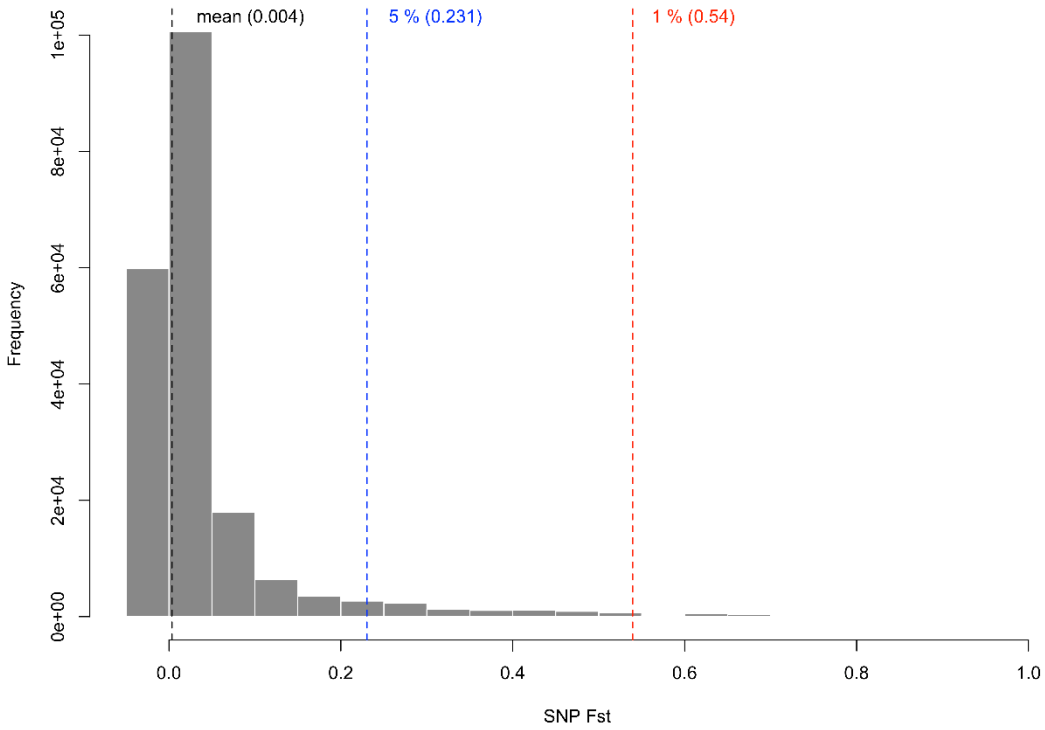

Figure 9. SNP F_{ST} distribution between relicts and non-relicts used in this study.

Mean as well as upper 5% and 1% tails were draw on the distribution with dash lines.

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Table 1. Information of 52 *A. thaliana* accessions

(A) Non-relicts

ID	Name	Longitude	Latitude	Country	Vernelization[a]	Fruit successfully[b]
10014	Xan-1	48.8	38.65	AZE		x
6986	Abd-0	-2.2207	57.1539	UK		
7013	Bd-0	13.287	52.4584	GER	x	
9761	Bik-1	35.7	33.92	LBN		
7127	Est	24.9871	58.6656	EST		
430	Gr-1	15.5	47	AUT		
7217	Lm-2	0.5	48	FRA	x	
7323	Rubezhnoe-1	38.28	49	UKR		
7394	Wa-1	21	52.3	POL	x	
6979	Wei-0	8.26	47.25	SUI	x	
9522	IP-Bea-0	-5.27	36.52	ESP		
9827	Bos-0	0.69	42.78	ESP		
7067	Ct-1	15	37.3	ITA		
6008	Duk	16.2	49.1	CZE	x	
9909	GEN-8	3.3	50.59	FRA	x	
9929	ISS-20	3.71	43.92	FRA	x	
6830	Kz-13	73.1	49.5	KAZ	x	
9857	Leg-0	-3.8	40.33	ESP		
6043	Löv-1	18.079	62.801	SWE		x
9727	Olympia-2	21.62	37.63	GRC		
9741	Orast-1	23.16	45.84	ROU		
9615	Parti-1	52.16	52.99	RUS		
9659	Pigna-1	14.18	41.18	ITA		
6096	T1060	13.2225	55.6472	SWE		x
5353	UKNW06-003	-3	54.5	UK		
7081	Co	-8.42639	40.2077	POR		
9571	IP-Pro-0	-6.01	43.28	ESP	x	
7316	Rhen-1	5.56667	51.9667	NED		
6981	Ws-2	30	52.3	RUS	x	
6909	Col-0	-92.3	38.3	USA	x	
6932	Ler-1	10.8719	47.984	GER	x	

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(B) Relicts

ID	Name	Longitude	Latitude	Country	Vernelization[a]	Fruit successfully[b]
6911	Cvi-0	-23.6167	15.1111	CPV		
9533	IP-Cem-0	-4.32	41.15	ESP		
9543	IP-Gra-0	-5.39	36.77	ESP		
9545	IP-Her-12	-5.78	39.4	ESP		
9549	IP-Hum-2	-3.69	42.23	ESP		
9554	IP-Lso-0	-3.16	38.86	ESP		x
9555	IP-Mar-1	-3.93	39.58	ESP		
9583	IP-Sne-0	-3.38	37.09	ESP		x
9598	IP-Vim-0	-6.51	41.88	ESP		
9600	IP-Vis-0	-6.04	39.85	ESP		
9762	Etna-2	14.98	37.69	ITA		
9764	Qar-8a	35.84	34.1	LBN		
9832	IP-Cat-0	-3.69	40.54	ESP		
9837	IP-Con-0	-5.6	37.94	ESP		
9871	IP-Nac-0	-3.99	40.75	ESP		x
9879	IP-Per-0	-1.12	37.6	ESP		
9905	IP-Ven-0	-4.01	40.76	ESP		x
9944	Don-0	-6.36	36.83	ESP		
9947	Ped-0	-3.9	40.74	ESP		x
	Tanz-1	36.12	-2.8739	TZ		
	Tanz-2	36.12	-2.8739	TZ		

a. “x” mark the accessions whose flowering time lower than three weeks and did not treat with vernerlization.

b. Accessions mark with “x” were exclude from measurements of seed-related traits.

doi:10.6342/NTU201801769

Table 2. Statistical analysis of shade avoidance response experiments.

(A) Tukey's honest significance test

Levels		Tukey HSD[a]		
Relict	R:FR			
Y	0.5	A		
N	0.5	A	B	
Y	2		B	C
N	2			C

a. Levels not connected by same letter are significantly different.

(B) Fixed effect tests

Source	DF	F Ratio	Prob > F
relict	1	1.2527	0.2719
treatment	1	474.1902	**<.0001**
relict × treatment	1	1.4223	0.2332

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Table 3. All traits of two populations measured in this study

Trait	Relict	Non-relict	*F*-value[a]	*P*-value[b]	Q_{ST}[c]
Hypocotyl length (mm) (R:FR = 2)	3.38 ± 0.34	3.32 ± 0.24	0.2446	0.6230	0.052
Hypocotyl length (mm) (R:FR = 0.5)	4.94 ± 0.34	4.54 ± 0.24	0.4419	0.5093	0
Seed number per fruit	31.78 ± 1.46	44.1 ± 1.08	45.6757	**<.0001**	0.783
Number of unfertilized ovule per fruit	6.31 ± 1.09	6.24 ± 0.8	0.0025	0.9604	0
Ovule number per fruit	38.09 ± 1.24	50.34 ± 0.92	62.6343	**<.0001**	0.819
Fertilized percentage	0.84 ± 0.02	0.88 ± 0.02	1.9239	0.1726	0.092
Fruit length (mm)	11.44 ± 0.31	11.22 ± 0.23	0.3171	0.5763	0
Average seed number per millimeter	2.78 ± 0.15	3.96 ± 0.11	41.9118	**<.0001**	0.700
Average ovule number per millimeter	3.37 ± 0.15	4.56 ± 0.11	42.0672	**<.0001**	0.722
Fruit number	54.75 ± 5.51	75.94 ± 4.06	9.5916	**0.0035**	0.482
Seed number	1741.62 ± 237.14	3378.53 ± 174.28	30.9369	**<.0001**	0.827
Ovule number	2155.25 ± 317.42	3957.64 ± 233.69	20.9096	**<.0001**	0.712
Seed size ($pixel^2$)	802.06 ± 24.25	601.76 ± 18.27	43.5064	**<.0001**	0.685
Seed length (pixel)	42.5 ± 0.85	35.78 ± 0.64	40.306	**<.0001**	0.668
Seed weight (pixel)	25.12 ± 0.36	22.58 ± 0.27	31.6973	**<.0001**	0.615

a. *F* value for the fixed relict effect

b. *P* value for the fixed relict effect

c. Q_{ST} estimating proportion of total genetic variation existing relicts and non-relicts

doi:10.6342/NTU201801769

Table 4. Fruit and seed-related traits of accessions treated with vernelization

Trait	Relict	Non-relict	*F*-value[a]	*P*-value[b]	Q_{ST}[c]
Seed number per fruit	31.79 ± 1.34	41.23 ± 1.31	25.4408	**<.0001**	0.726
Number of unfertilized ovule per fruit	6.31 ± 1.11	6.92 ± 1.08	0.1564	0.6953	0
Ovule number per fruit	38.10 ± 1.12	48.16 ± 1.10	41.0774	**<.0001**	0.807
Fertilized percentage	0.84 ± 0.02	0.86 ± 0.02	0.3922	0.5358	0
Fruit length (mm)	11.43 ± 0.31	11.16 ± 0.31	0.4038	0.5299	0
Average seed number per millimeter	2.78 ± 0.14	3.73 ± 0.14	22.9431	**<.0001**	0.613
Average ovule number per millimeter	3.37 ± 0.15	4.40 ± 0.15	22.7833	**<.0001**	0.627
Fruit number	54.76 ± 5.11	79.50 ± 4.97	12.0460	**0.0016**	0.647
Seed number	1743.12 ± 218.71	3363.45 ± 211.87	28.3158	**<.0001**	0.939
Ovule number	2156.98 ± 265.88	3966.00 ± 258.03	20.9096	**<.0001**	0.864
Seed size ($pixel^2$)	802.06 ± 25.57	628.62 ± 25.48	23.0915	**<.0001**	0.590
Seed length (pixel)	42.5 ± 0.89	36.79 ± 0.89	20.6593	**<.0001**	0.560
Seed weight (pixel)	25.12 ± 0.37	22.88 ± 0.37	18.5453	**0.0002**	0.537

a. *F* value for the fixed relict effect

b. *P* value for the fixed relict effect

c. Q_{ST} estimating proportion of total genetic variation existing relicts and non-relicts

doi:10.6342/NTU201801769