

Effects of the Two-early-flowering-time Genes, *Ef1* and *m-Ef1*, Founded in Low Latitude were Reexamined in One of the Origins of Donor Parent under Fluctuating Field Environments as to Earliness Effect and Fitness

Youki Itoh^{1*}

¹Laboratory of Wildlife Ecology, Department of Environmental Symbiotic Science, College of Agriculture, Food and Environmental Sciences, Rakuno Gakuen University, Japan.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAERI/2015/18360

Editor(s):

(1) Abdel-Tawab H. Mossa, Environmental Toxicology Research Unit (ETRU), Pesticide Chemistry Department, National Research Centre, Egypt.

Reviewers:

(1) Rezzoug Waffa, Ibn Khaldoun University, Algeria.

(2) Klára Kosová, Crop Research Institute, Prague, Czech Republic.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=1187&id=37&aid=9415>

Original Research Article

Received 17th April 2015
Accepted 1st May 2015
Published 25th May 2015

ABSTRACT

Aims: Early flowering 1 (*Ef1*) and modifier of Early flowering 1 (*m-Ef1*) genes were firstly detected in the cultivars fitted to northern margins of rice cultivation. Hence, it has been inferred that the genes might be essential for adaptation to the regions. However, the genes have been identified in low latitudes and the effects on earliness and fitness in the origins have not been tested. To know genetic shift in response to climatic change, the present study was performed as to earliness effect and fitness.

Study Design: The six near-isogenic lines (NILs) associated with *Ef1* and *m-Ef1* genes and the recurrent parent were used. In each genotype, twelve plants were divided into two replicates in a paddy field. In each replicate, genotypes were placed at random.

Place and Duration of Study: The place of this study was National Agricultural Research Center in Sapporo (43 °N) in Japan. Duration of the study was summer season between year 2003 (cool

*Corresponding author: Email: yoshiemetkoneko@hotmail.co.jp;

summer) and 2004 (warm summer).

Methodology: In each plant, flowering time was recorded every day and days to flowering from germination to (DF) were calculated. Earliness effect of a plant of a NIL was defined as subtraction of DF of a NIL from that of the recurrent parent in same ID of same replicate. After harvest, maximum fertility of a panicle (F_{\max}), minimum fertility of a panicle (F_{\min}), total number of panicles with more than a fertile grain (Effective panicle number: EPN) and total number of panicles (TNP) were recorded in each plant. Fitness in a plant was conventionally estimated from the formulation, $1/2 (F_{\max} + F_{\min}) \times (EPN / TNP)$.

Results: Compared with the previous study, which evaluated the effect of *Ef1* and *m-Ef1* genes in low latitude, effect of epistasis between *Ef1* and *m-Ef1* on earliness qualitatively changed between low and high latitudes. Furthermore, it was estimated that one of the epistasis might accelerate flowering time in response to low temperatures. At phenotype level, Individuals with earlier flowering time tended to show higher fitness. However, at genotype level, the tendency was broken in low-temperature year. As a result, correlation of fitness between two years showed no significance.

Conclusion: Epistasis-by-environment interaction might reveal significant role in earliness effects between latitude and between years. In addition, even in the case that earliness effects were preserved among genotypes between years, there is a possibility that fitness might not always be conserved under fluctuating field conditions in the northern limit of rice cultivation.

Keywords: Climatic change; epistasis; fitness; flowering time; genotype-by-environment interaction; rice cultivation; Sapporo.

1. INTRODUCTION

Farmers use phenotypes while plant breeders change genotypes. A genotype often changes the effect on phenotype in response to environments [1,2]. Hence, pursuit of alternations of genotype-phenotype relationship along the ecological context is one of the most important topics in agronomical study. For examining the relationship, flowering time is the preferable trait since flowering time is easily measured adaptive phenotype in higher plant [3,4]. In the most northern regions of rice cultivation, cultivars generally show an early flowering behavior. The two genes, *Ef1* (*Early flowering 1*) and *m-Ef1* (*modifier of Early flowering 1*), were firstly detected in two landraces grown in the northern areas of Japan and China. It has been reported that *Ef1* shortens the duration to floral initiation from germination without changing its photoperiod sensitivity, and *m-Ef1* intensifies the earliness due to *Ef1* in Taiwan (24°N) [5]. *Ef1* and *m-Ef1* showed an epistasis, which contributed to earliness in Taiwan, implying that the epistasis as well as an additive effect might play a significant role for adaptability to the northern margins of their distributions. However, the manner of functional relationship between *Ef1* and *m-Ef1* genes has not been tested in their origins. Then, the first question arises as follows: Can we suppose that the contribution manners of genes to earliness effect might be qualitatively preserved between different locations? This

question is also associated with application of quantitative locus analyses, which have recently come into fashions, to plant-breeding program.

In the environment of northern margins of rice cultivation, the two features are remarkable as to temperature change. First, the temperature shows a large difference between years [6,7, see also Fig. 1] Then, we can examine how the differences of year environments change genotype-phenotype relationship in the region [2] (the second question). Second, during cultivation period, temperature drastically increases and decreases in a short span and the difference between maximum and minimum temperatures in the region are equivalent to that between different latitudes. Hence, adequate duration of flowering time is highly limited for obtaining a stable yield [8, see also Fig. 1]. It has been thought that flowering time is agronomically important in rice because this trait is strongly associated with the regional adaptability and fitness [9]. However, fitness is composed of various component traits, which interact each other and change their effects, depending on environmental conditions [2,10,11]. Therefore, to strictly demonstrate the thought, it is the best way that relationship between earliness effect and fitness is examined using flowering-time near-isogenic lines (NILs) in a northern-limit region of rice cultivation where the temperature largely changes in a cultivation period (the third question).

If earliness as phenotype significantly affects fitness, then the last question arises as follows: Can we expect that the flowering genotypes might stably contribute to fitness in the origin of donor parent? When flowering-time NILs are examined as to fitness in the field environments, this question can be easily resolved.

Here, objectives of the present study are answering to the four questions using flowering-time NILs in order to predict genetic shifts in response to climatic changes. That is, first, I examined whether or not contribution manner of *Ef1* and/or *m-Ef1* to flowering time in low latitude is same as that in high latitude in field environment. Second, I examined whether or not *Ef1* and/or *m-Ef1* respond to the difference of year environment in Sapporo. Third, I intended to quantify how degree earliness effect contributes to fitness at phenotype level in Sapporo. Last, I examined whether or not order of flowering-time genotype in the contribution to fitness is preserved under fluctuating field environments if flowering time as a phenotypic trait contributes to fitness across years in Sapporo.

2. MATERIALS AND METHODS

2.1 Genetic Materials

A total of seven strains (six NILs and the recurrent parent) were used in the present study (Table 1). NILs were made by successive backcrossing method and the backcross generation is 20 [5]. The recurrent parent, T65, was derived from Taichung 65 and is a practically photoperiod insensitive strain [5,12]. The NILs were conveniently divided into two groups, T65NILs A and T65NILs B. *Ef1^a* and/or *m-Ef1^a* involved in T65E^a, T65m^a and T65E^am^a which belongs to T65NIL A were originated from Tatong-tailai which is fitted to northern China. While *Ef1^b* and/or *m-Ef1^b* involved in T65E^b, T65m^b and T65E^bm^b which belongs to T65NIL B were originated from Bozu 5 which is fitted to northern Japan (Hokkaido islands). Recently, *Ehd1* (*Early heading date-1*), which encodes B-type response regulator, has been proposed to a candidate for *Ef1* since amino acid substitution (Arg to Gly) in exon 4 of *Ehd1* promoted flowering in the genetic background of T65, and the gene was located on chromosome 10 as is *Ef1* [13]. Uwatoko et al. [12] confirmed the proposition based on DNA polymorphism, using T65E^b and the donor parent, Bozu 5. *m-Ef1* is located on chromosome 7 [14] and the dominant

allele clearly showed photoperiod sensitivity when it was combined with *Ef1* although *m-Ef1* has not yet been characterized at molecular level.

2.2 Cultivation and Trait Evaluation

The way of cultivation was summarized in Table 2. Seeds were germinated in petri-dishes in the dark (30°C) in three days and each of the seedlings raised in a hole (1.5 cm diameter) with culture soil of seed-bed. The seedlings were grown in a greenhouse at Hokkaido Agricultural Research Center (HARC) in Sapporo (43°N) until transplanting time. Selected seedlings of each genotype were transplanted in an irrigated paddy field at HARC. In the paddy field, genotypes were placed at random in each of the two replicates in year 2003. In year 2004, genotypes were transplanted in the same area of the same paddy field and the arrangement of genotypes in each of the replicates was same as that of year 2003. It is noted that in year 2003 and 2004 there was cool and very warm summer, respectively (Fig. 1). In year 2003, modern cultivars highly fitted to Hokkaido islands were subjected to cool weather damage. On the other hands, in year 2004, T65 fitted to Taiwan showed a level of fertility. To avoid skewing the estimation of earliness effect on fitness, rice plants were sprayed with an insecticide when panicles emerged. From the same reason, at a month after transplanting, herbicide was used for avoiding competition against weeds.

In each plant, the first date of panicle emergence was observed everyday and the date was regarded as flowering time. Earliness effect of each plant of a NIL was defined as subtraction of flowering time of a NIL from that of the recurrent parent in same ID in same replicate. After harvest, maximum fertility of a panicle (F_{max}), minimum fertility of a panicle (F_{min}), total number of panicles with more than a fertile grain (Effective panicle number: EPN) and total number of panicles (TNP) were recorded in each plant. Fitness in a plant was conventionally estimated from the formulation, $1/2 (F_{max} + F_{min}) \times (EPN / TNP)$ since fitness was mostly affected by fertility in the northern margin of rice cultivation [8]. If all the panicles show fertility at 100% level, fitness results in 1.00. If all the panicles do not have fertile grain, fitness results in 0.00. It is noteworthy that fitness does not always reflect yield. All the statistical tests in the present study were carried out using Stat View 5.0 [15].

Table 1. Near-isogenic lines (NIL) of Taichung 65 (T65) used in the present study

Strain	Gene involved ¹⁾		BC ²⁾	Donor parent	Days to heading in year	
	<i>Ef1</i>	<i>m-Ef1</i>			2003	2004
<T65NILs A>						
T65E ^a	<i>Ef1</i> ^a	+	20	Tatong-tailai	132.5±1.2	116.5±2.5
T65m ^a	+	<i>m-Ef1</i> ^a	20	Tatong-tailai	143.0±1.7	123.3±2.2
T65E ^a m ^a	<i>Ef1</i> ^a	<i>m-Ef1</i> ^a	20	Tatong-tailai	121.4±1.7	105.0±1.3
<T65NILs B>						
T65E ^b	<i>Ef1</i> ^b	+	20	Bozu 5	133.3±1.6	115.9±1.8
T65m ^b	+	<i>m-Ef1</i> ^b	20	Bozu 5	142.3±1.8	122.9±2.5
T65E ^b m ^b	<i>Ef1</i> ^b	<i>m-Ef1</i> ^b	20	Bozu 5	130.2±1.4	111.0±1.0
<Recurrent parent>						
T65 ³⁾	+	+	-	-	146.1±1.8	126.6±2.8

¹⁾ + indicates wild allele. ²⁾ BC indicates backcross generation. ³⁾ Tatong-tailai and Bozu 5 is a cultivar originated from northern China and Japan (Hokkaido islands), respectively. ⁴⁾ T65 is a pure line of Taichung 65 (progeny of a self-pollinated single plant)

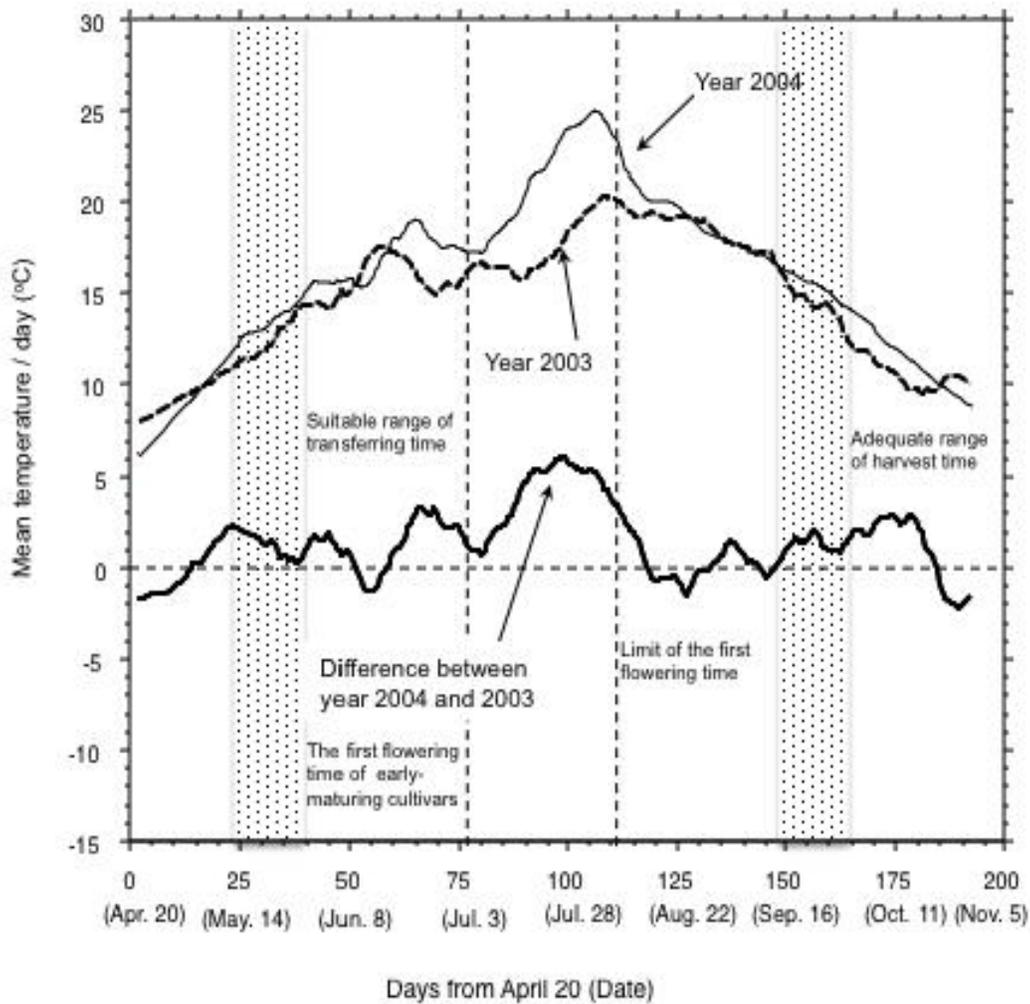


Fig. 1. Changes in daily mean air temperature from sowing to maturation of grain in year 2004 and 2003 and their difference in Sapporo (43°N)

Table 2. Cultivations and year environments in the present study

Number of plant examined	Year 2003	Year 2004
	Six plants (/ a replicate) x two replicates / a strain	
Date of germination	April 19	April 21
Date of sowing	April 21	April 24
Date of transfer	May 22	May 19
Density	35-cm x 12.5-cm	35-cm x 12.5-cm
Fertilizer	1kgN-1.2kgP-0.86kgK / a	1kgN-1.2kgP-0.86kgK / a
Limit period of cultivation	October 31	October 7
Year type (See Fig. 1)	Cool summer	Very warm summer

3. RESULTS AND DISCUSSION

3.1 Comparison of Functional Manner of *Ef1* and *m-Ef1* Genes between Low and High Latitudes

All the NILs significantly flowered earlier than the recurrent parent in Sapporo (Table 1; Fig. 2), NILs with *Ef1* genes (i.e. T65E^a and T65E^b) flowered earlier than NILs with *m-Ef1* genes (i.e. T65m^a and T65m^b) and NILs with both of the two genes (i.e. T65E^am^a and T65E^bm^b) flowered earlier than NILs with *Ef1* genes in Sapporo. The order of earliness in Sapporo is nearly consistent with that in Taichung (Fig. 2). However, there are three differences as to functional manner of *Ef1* and *m-Ef1* genes between Taichung and Sapporo. First, *m-Ef1* genes did not show earliness effects in the summer crop season in Taichung, while *m-Ef1* genes stably showed significant earliness effects across years in Sapporo. Considering small range of suitable flowering time in the northern-limit region of rice cultivation [16, see also Fig. 1], the result indicates that *m-Ef1* genes are essential for earliness in the field environments although the effects were small. Second, allelic difference between *Ef1*^a and *Ef1*^b was detected in Taichung while the difference was not detected in Sapporo. One may think that allelic difference is a genetic feature which is independent on the environment. It seems to me that such thought may occur due to lack of reaction norm concept (e.g., photoperiodic response curve). The fact that the allelic difference in a low latitude disappeared in a high latitude indicates that each of *Ef1* alleles showed genotype-by-environment interaction since these genetic materials are NILs of *Ef1*. Such case is not novel phenomenon especially in regulatory genes [1]. For instance, *photosensitivity 1* (*Se1*) alleles showed different flowering times in a range of photoperiodic conditions but under a photoperiodic condition, the two alleles showed no difference in flowering

time [17]. The third difference is associated with epistasis between *Ef1* and *m-Ef1*. Epistasis between *Ef1*^a and *m-Ef1*^a contributed to lateness while that between *Ef1*^b and *m-Ef1*^b contributed to earliness in Taichung [5]. On the other hands, epistasis was detected only between *Ef1*^a and *m-Ef1*^a in Sapporo (Table 3). In addition, epistasis between *Ef1*^a and *m-Ef1*^a contributed to earliness in Sapporo (Fig. 2) Tsai reported that the interaction between *m-Ef1*^b and *m-Ef1*^b (probably the first detected epistasis in rice) was emphasized, and it has been implied that the effect of epistasis on earliness might be essential for adaptation to the northern margin of rice distribution (or Hokkaido islands) [5]. In addition, Uwatoko et al. [14] also identified the significant effects of epistasis between *m-Ef1*^b and *Ef1*^b on the components of photoperiodic reaction norm in growth-cabinet experiment. However, the epistasis was not detected in both year 2003 and 2004 in the field environments of Sapporo (Table 3), suggesting importance of examination of genic contribution in their origins or regions where plant-breeding programs are performed. The interaction between *Ef1*^b and *m-Ef1*^b might occur under high-temperature condition since Uwatoko et al. adopted so high-temperature condition as the growth resume and the temperatures in Taiwan is higher than that in Sapporo.

Wright emphasized the importance of epistasis in phenotypic variation, which is the material of evolution but it is likely that relationship between epistasis and environments was not kept in his mind [18]. Recently, it has been reported that epistasis associated with fitness-related traits interacted environment in rice and *Arabidopsis* [14,19], suggesting that emergence of variation of epistasis might depend on environmental context. In this sense, epistasis will contribute to enlargement of phenotypic variation, which can easily induce a novel phenotype under a new environment. The present result directly indicates this concept.

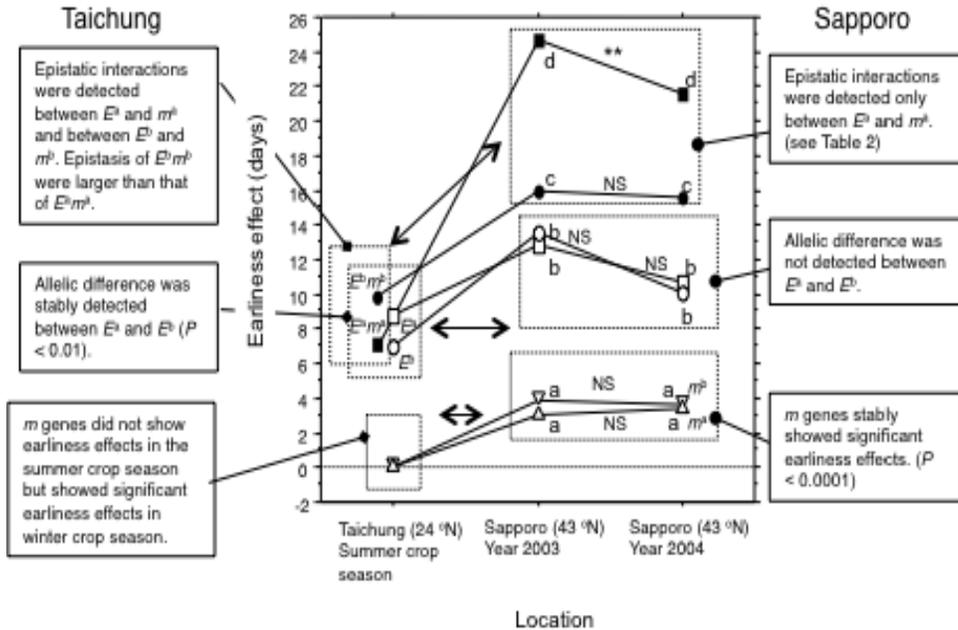


Fig. 2. Comparisons of earliness effect of the six NILs of Taichung 65 (BC20 generation) between Taichung and Sapporo and between years 2003 and 2004 in Sapporo

Earliness effect of a NIL was calculated by subtracting days to flowering of a NIL from that of the recurrent parent. Data of Taiwan were roughly estimated from Fig. 1 of reference [5]. E and m indicates $Ef1$ and $m-Ef1$, respectively. In Sapporo, same letters (i.e. a, b, c and d) indicate that the differences of earliness effects between the strains are not significant in each year (Tukey HSD-test). All the earliness effects of the genes were significant at 0.1% levels. In Sapporo, difference of earliness effect of a NIL between years was tested by t -test. ** and NS indicates significance at 1% level and non-significance ($P > 0.05$), respectively

3.2 Response of Function of $Ef1$ and $m-Ef1$ Genes to Year Environments

There were large differences in temperature between year 2003 and 2004 in Sapporo (Fig. 1). Nevertheless, all the NILs did not show year response as to the earliness effects except for $T65E^a m^a$ (Fig. 2). Both $Ef1^a$ and $m-Ef1^a$ genes did not show year response. Hence, the significant year response shown by $T65E^a m^a$ might be derived from epistasis-by-year environment interaction. It is noted that $T65E^a m^a$ made earliness effect larger in cool year than in warm year, suggesting the epistasis might increase the earliness effect in response to low temperatures. This suggestion is also supported by the phenomenon that combination of $Ef1^a$ with $m-Ef1^a$ gene contributed to lateness in Taichung where temperatures are high while that contributed to earliness in Sapporo where temperatures are low (Fig. 2). In the northern-limit region of rice cultivation, suitable range of flowering time is highly limited because cultivation period is mostly covered with low temperatures (Fig. 1). In such situation, two

strategies of flowering time are thought. First, when high temperatures are maintained in the early stage of flowering time, it is advantageous for rice plant to accelerate flowering because the rice plants, which cannot have strong photoperiod sensitivity due to the long days in summer time [16], cannot predict the timing when low temperatures occur and there is the capricious timing of occurrence of low temperature in the region [7]. A gene associated with the first strategy was identified in a land race of Hokkaido, and hence, the genetic mechanism for the strategy would exist [20,21]. The epistasis between $Ef1^b$ and $m-Ef1^b$ might belong to the first strategy. Second, when low temperatures are maintained in the late stage of flowering time, it is advantageous for rice plant to accelerate flowering to avoid cool weather damage. However, genetic mechanism for the second strategy has not been identified. The present result might provide a piece of genetic evidence with the second strategy, that is, epistasis between $m-Ef1^a$ and $m-Ef1^a$ might contribute to earliness in response to low temperature.

3.3 Relationship between Earliness Effect and Fitness

Takahashi [8] mentioned that extension of rice cultivation toward the most northern areas has owed to mainly genetic improvements of earliness and cool tolerance of booting stage. However, it has not been quantitatively shown how degree earliness affects fitness since plant breeders have genetically improved both earliness and cool tolerance of booting stage. T65 is a weak strain as to cool tolerance of booting stage [22], and hence, the earliness NILs with the genetic background of T65 are suitable for examining the pure effect of earliness on fitness. At individual level, earliness effects significantly affected fitness in both year 2003 and 2004 in Sapporo (Fig. 3). The present study revealed that earliness effect as phenotype explained at least 51.8% of total variation of fitness (Fig. 3). This is the first depiction of the relationship between earliness effect and fitness as to the quantitative viewpoint. Individual with earlier flowering time tended to show higher fitness, suggesting early-flowering time is essential component of fitness in the northern-limit region of rice cultivation. R^2 value of year 2003 was smaller than that of year 2004, suggesting that low temperatures might disrupt linear relationship between earliness effect and fitness.

The fitness values at genotype level tended to greatly fluctuate depending on year and

correlation of the fitness between year 2003 and 2004 was not significant ($r = 0.182$) (Fig. 4). At genotype level, the fitness values were significantly correlated with the earliness effects in year 2004 ($r = 0.886$, $P < 0.05$), however, the tendency disappeared in year 2003 ($r = 0.725^{\text{NS}}$) (Fig. 4). Nevertheless, the earliness effect of six genotypes showed similar range between the two years, showing highly significant correlation ($r = 0.986$, $P < 0.0001$) (Fig. 4). This seems to be caused by a lower value of fitness in the genotypes with $Ef1^a$ *m-Ef1^a* or $Ef1^b$ *m-Ef1^b* than those with $Ef1^a$ or $Ef1^b$ alone in year 2003. After time exceeds the limit of suitable range of flowering time, which has been experienced by many rice breeders in the region, environments are averagely covered with low temperatures (Fig. 1) but capricious high temperatures in only several hours in a day can often occur in the northern-limit region of rice cultivation. This may be the cause for the confusion of linear relationship between earliness effects and fitness at genotype level. Whatever the reason, it can be at least said that year environments qualitatively affected the contribution manner of genotype of flowering time (or a fitness component trait) to fitness. This is a very commonplace experience of the rice breeders in the northern margins of rice cultivation, but there has been no scientific depiction that directly shows the experience. Therefore, Fig. 4 provides a foundation with future plant breeding studies and can be a ground for the reason why a rice-breeding program has required many years in the region.

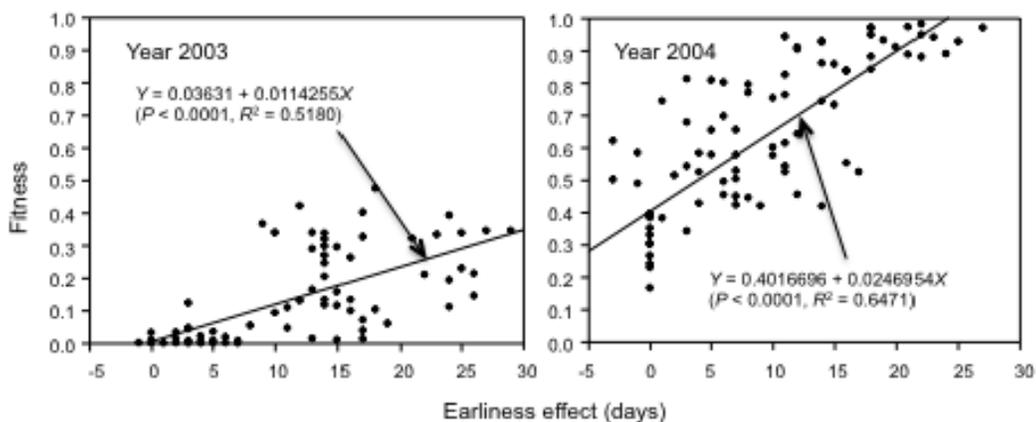


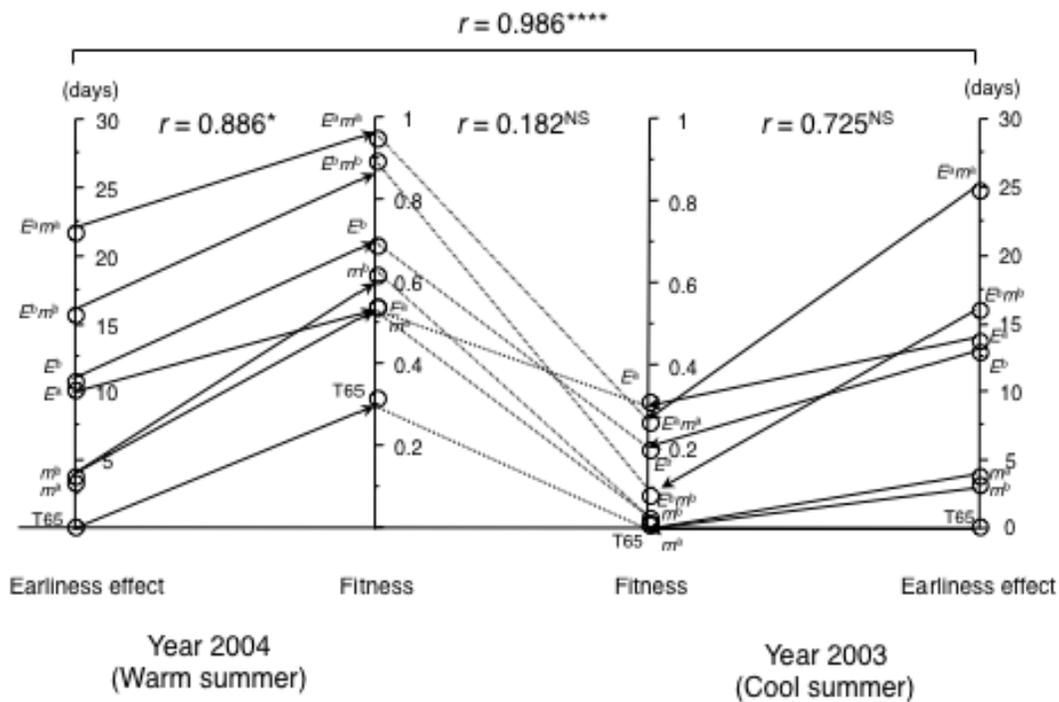
Fig. 3. Relationship between earliness effect and fitness at individual level

Earliness effects of a plant of a NIL were calculated by subtracting days to flowering from germination of a NIL from that of the recurrent parent in same ID of same replicate. Thus, earliness effect of the recurrent parent is zero. Fitness in a plant was estimated from the formulation, $1/2$ (Maximum fertility of a panicle + Minimum fertility of a panicle) \times (Total number of panicles with more than a fertile grain / Total number of panicles)

Table 3. Mean squares of days to flowering from germination for *Ef1* and *m-Ef1* loci within each of years

Source	df	NILs A ¹⁾		NILs B	
		Year 2003	Year 2004	Year 2003	Year 2004
<i>Ef1</i>	1	3383.521**** ²⁾	2408.333****	1633.333****	1530.021****
<i>m-Ef1</i>	1	475.021****	660.083****	85.333**	221.021****
<i>Ef1</i> x <i>m-Ef1</i>	1	275.521****	200.083****	2.083 ^{NS}	4.688 ^{NS}
Residual	44	10.203****	5.231****	10.345*	4.608****

¹⁾ In the NILs A, ANOVA was carried out by using the values of $T65E^a$, $T65m^a$, $T65E^am^a$ and $T65$, while in NILs B, ANOVA was carried out by using the values of $T65E^b$, $T65m^b$, $T65E^bm^b$ and $T65$. ²⁾ **, **** and ^{NS} indicates significance at 1%, 0.01% levels and non-significance, respectively

**Fig. 4. Relationships between earliness effects and fitness at genotype level in the years 2003 and 2004, and their comparison in Sapporo**

Earliness effects of a NIL were calculated by subtracting mean of days to flowering of a NIL from that of the recurrent parent. Fitness in a plant was estimated from the formulation, $1/2$ (Maximum fertility of a panicle + Minimum fertility of a panicle) \times (Total number of panicles with more than a fertile grain / Total number of panicles). One-tailed correlation coefficients (r) were calculated by using genotypic mean. *, ****, and ^{NS} indicates significance at 5 and 0.01 % levels and non-significance, respectively

4. CONCLUSION

Genic effects of *Ef1* and *m-Ef1* on flowering time largely changed between different latitudes. Especially, epistasis between the two genes qualitatively changed, suggesting that the role of epistasis in emergence of novel phenotype in response to environments should not be negligible. In addition, epistasis between $Ef1^a$ and $m-Ef1^a$ may respond to low temperatures and accelerate flowering time. This is the first

suggested phenomenon in rice. Using flowering-time NILs, it has been firstly demonstrated that earliness effect as phenotype (a phenotypic trait) significantly affects fitness in both cool and warm years in one of the northern-limit regions of rice cultivation. Individual with earlier flowering time tended to show higher fitness. However, this tendency was not always preserved at genotype level. Capricious changes of temperature might disrupt the tendency and this shows the difficulty of plant breeding programs in the region.

ACKNOWLEDGEMENTS

A paddy field was provided for the present study by Mr. Hiroyuki Shimizu (HARC). The use of genetic materials was permitted by professor Yoshio Sano (Research Group of Breeding Science, Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University). Y. Itoh thanks Miss Yoshie Sato (GASUTO, Fukuzumi) and Dr. Hiroshi Yorozuya (Brown Bear Research Group of Hokkaido University) for their encouragement and discussions about the present study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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