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Effects of barley chromosome addition to wheat on behavior and development of *Locusta migratoria* nymphs

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Locusta migratoria **feeds on various Poaceae plants but barley. Barley genes related to feeding deterrence may be useful for developing novel resistant crops. We investigated the effects of barley cultivar Betzes, wheat cultivar Chinese Spring (CS), and six barley chromosome disomic addition lines of wheat (2H–7H) on locomotor activity, feeding behavior, survival and development of** ***L. migratoria*** **nymphs. Locomotor activity was similar in nymphs kept with wheat and 2H–7H in an actograph, whereas it was generally high in those kept with barely. No-choice and choice feeding tests suggested that barley genes related to inhibition of feeding by** ***L. migratoria*** **are located on barley chromosomes 5H and 6H and those related to the palatability of plants on chromosomes 2H, 5H and 6H. Rearing experiments suggested the presence of barley genes negatively affecting the survival and growth of locust nymphs on chromosomes 5H and 2H, respectively, and the effects are phase-dependent.**

Several species of locusts including the migratory locust *Locusta migratoria* L. (Orthoptera: Acrididae: Oedipodinae) and the desert locust *Schistocerca gregaria* Forskål (Acrididae: Cyrtacanthacridinae) are potentially the most destructive agricultural pest insects worldwide. A notable trait of these species is the exhibition of density-dependent phase polyphenism, involving graded changes in behavioral, morphological and physiological traits[1–5].

Among these locusts, *L. migratoria* is distributed widely from tropical to subarctic regions of the Old World[6,7] and often undergoes outbreaks[8–11]. Although the locust feeds on various Poaceae including important crops such as rice *Oryza sativa* L., sorghum *Sorghum bicolor* (L.) Moench and wheat *Triticum aestivum* L., previous studies reported that it does not feed on barley *Hordeum vulgare* L. (Poaceae)[12,13]. These observations strongly suggest that barley possesses certain factors inhibiting feeding by *L. migratoria*. Identifications of such antifeeding factors will be useful for creating novel resistant varieties of cereal crops against the migratory locust. A previous study reported that gramine, the principal alkaloid in barley leaves[14], is partially related to the feeding deterrence of barley against *L. migratoria*[13]. However, the level of gramine alone did not fully account for the deterrence of barley against the locust, suggesting the involvement of multiple factors[13].

Utilizing the high cross compatibility of *T. aestivum*, several wheat-barley chromosome addition lines have been established: for example, *H. vulgare* cv. Betzes chromosome disomic addition lines of *T. aestivum* cv. Chinese Spring was produced[15] and *H. vulgare* cv. New golden chromosomes 5 and 6 were added to *T. aestivum* cv. Shinchunaga[16]. Such chromosome addition lines are useful to clarify the barley genes related to the resistance against herbivores[17].

To clarify barley chromosomes possessing resistant genes against the migratory locust, wheat cultivar Chinese Spring (CS, hereafter), barley cultivar Betzes, and six Betzes chromosome disomic addition lines of CS (2H–7H) were used in this study. We investigated locomotor activity in hatchlings of *L. migratoria* kept with wheat, barley and wheat-barley chromosome addition lines in an actograph, and examined the effects of barley chromosome addition to wheat on feeding acceptability, preference, survival and growth of *L. migratoria* nymphs.

In *L. migratoria*, adults grown at high population densities (gregarious phase) produce larger offspring than those grown at low population densities (solitarious phase) and gregarious and solitarious hatchlings can be

obtained by rearing locusts in group and in isolation, respectively, in the laboratory[1]. In the present study, we used hatchlings derived from adults reared in group and in isolation to determine if phase would affect feeding and developmental performance in this locust.

Results

Locomotor activity in *L. migratoria* nymphs kept with wheat, barley and barley chromosome addition lines of wheat. In the experiment using CS, Betzes, and filter paper, locomotor activities of hatchlings kept with Betzes and filter paper, respectively, gradually decreased from 1 to 18 hours and were not significantly different from one another throughout the experiment (ANOVA followed by Tukey's HSD test; Fig. 1). Although hatchlings kept with CS were similarly active as the others at the beginning (1 h) of experiment, they exhibited a significantly lower level of activity than those kept with Betzes and filter paper. The difference was significant between CS and Betzes treatments from 2 to 11 hours and between CS and filter paper treatments from 5 to 10 hours (ANOVA followed by Tukey's HSD test; $p < 0.05$). Locomotor activity of the hatchlings in the CS treatment gradually increased from 10 to 14 hours and became significantly higher than that in the filter paper treatment at 14 hours or later (ANOVA followed by Tukey's HSD test; $p < 0.05$).

In the experiment using wheat-barley chromosome addition lines, no significant differences were detected between the CS control and any of the 2H–7H treatments except at 10 hours when locomotor activity in the 2H treatment was significantly higher than that in the control ($p < 0.01$; Dunnet's test; Fig. 2). As observed in the previous experiment (Fig. 1), locomotor activity of the hatchlings kept with Betzes was significantly higher than that in the control from 5 to 13 hours ($p < 0.05$ at 13 hours, $p < 0.01$ for 11 hours, and $p < 0.001$ for others; Dunnet's test; Fig. 2).

Effect of barley chromosome addition to wheat on feeding preference of *L. migratoria*. When hatchlings were kept with one of 2H–7H, Betzes, or CS (no-choice test), no significant differences were detected among treatments and control in the amount of leaves consumed by locust hatchlings at 1 and 4 hours (Fig. 3). After 24 hours, however, hatchlings given 5H and 6H ate significantly smaller amounts of leaves than those given CS (Dunnet's test; $p < 0.05$; Fig. 3).

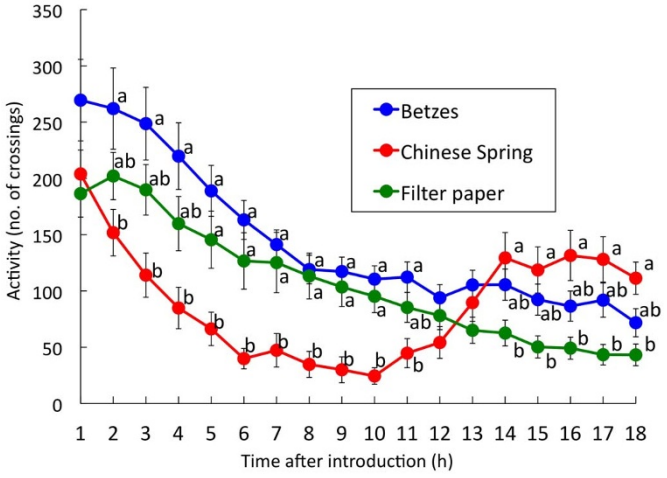

Figure 1 | Locomotor activity patterns of *Locusta migratoria* nymphs (gregarious phase, Iheya strain) kept with barley leaves (cv. Betzes), wheat leaves (cv. Chinese Spring) or wet filter paper. One-day-old hatchlings were individually introduced to actograph. Different letters indicate significant differences between treatments at each time (ANOVA followed by Tukey's HSD test; $p < 0.05$). Bars indicate SE.

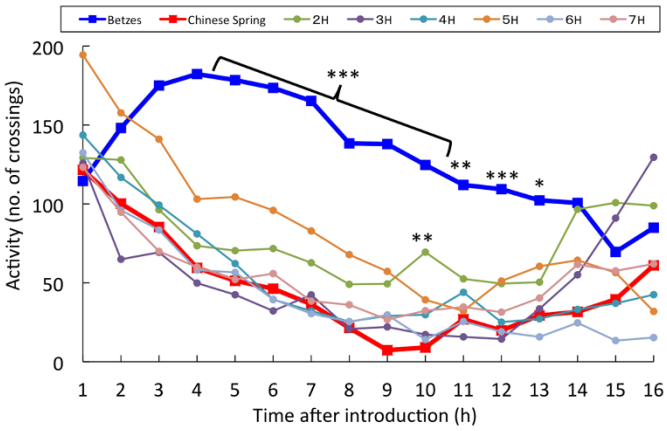

Figure 2 | Locomotor activity patterns of *Locusta migratoria* nymphs (gregarious phase, Iheya strain) kept with barley leaves (cv. Betzes), wheat leaves (cv. Chinese Spring) or leaves of one of six Betzes chromosome disomic addition lines (2H–7H) of Chinese Spring (see text for details). One-day-old hatchlings were individually introduced to actograph. Asterisks indicate significant differences from control (Chinese Spring) at each time (Dunnet's test; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$). Error bars are omitted from the figure for clarity but shown in Supplementary Table S1.

When one of 2–7H was presented together with CS to hatchlings (choice test), no significant difference was detected between any of the test plants and CS control in the total amount of leaves consumed during the first 4, 8, and 24 hours of observations. However, 2H, 5H and 6H were consumed significantly less than CS when compared at 44 hours (ANOVA, $p < 0.05$; Fig. 4).

Effects of barley chromosome addition on feeding, survival and growth in gregarious and solitarious *L. migratoria* nymphs. Gregarious nymphs, which were derived from crowd-reared parents, consumed less leaves when provided with Betzes versus CS (chi square test; $p < 0.01$; Table 1). Nymphal survival rate was significantly lower in the Betzes treatment than in the CS control (chi square test; $p < 0.001$; Table 1). The duration of the 1st instar was longer in the Betzes treatment than in the CS control ($p < 0.001$;

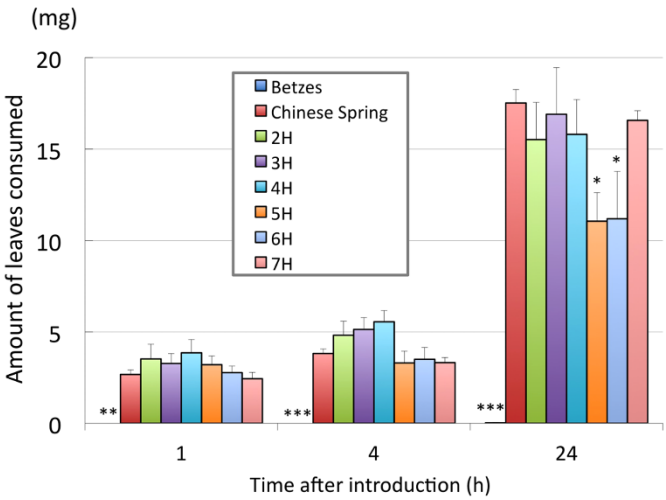

Figure 3 | The amount of leaves (mg) consumed by gregarious phase hatchling of *Locusta migratoria* (gregarious phase, Iheya strain) within 1, 4, and 24 hours (no-choice test). Asterisks indicate significant difference between control (CS) and treatment (Dunnet's test; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$). Bars indicate SE.

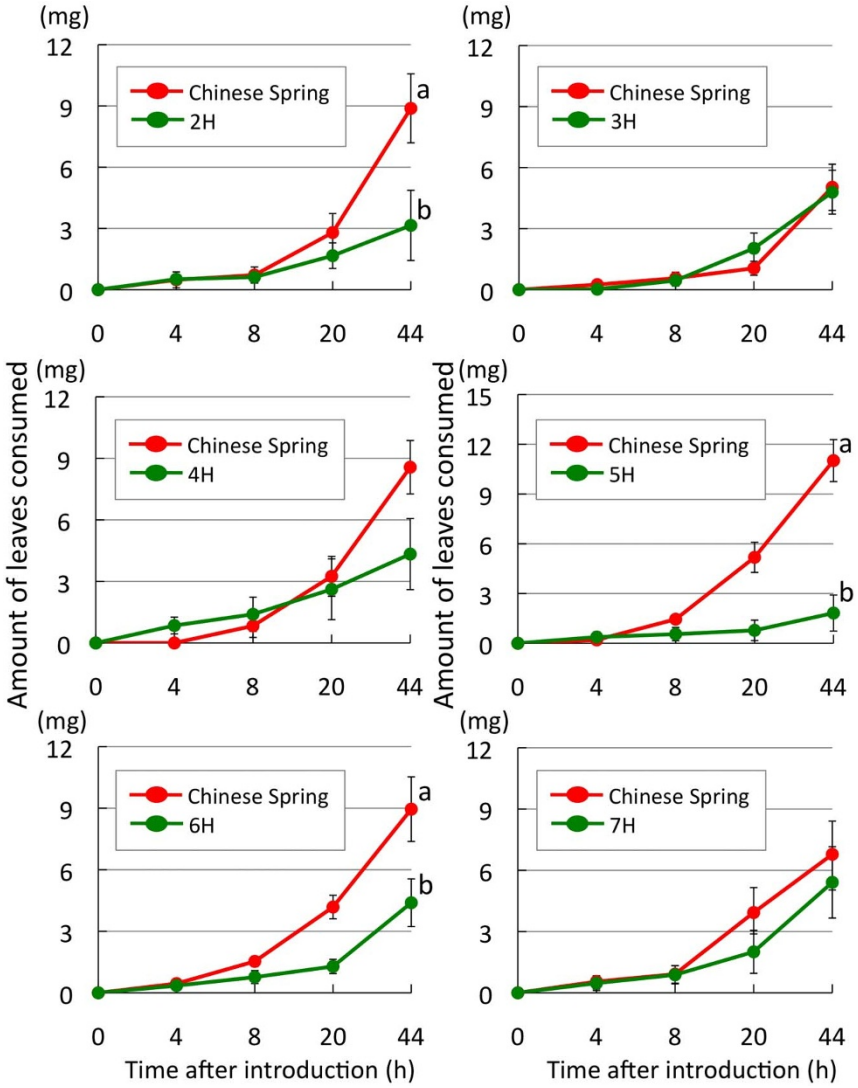

Figure 4 | The amount of leaves consumed by hatchlings of *Locusta migratoria* (gregarious phase, Iheya strain) within 4, 8, 20, and 24 hours (choice test). Abbreviations are as follows: CS, wheat cultivar 'Chinese Spring'; 2H–7H, Betzes chromosome disomic addition lines of Chinese Spring (see text for details). Different letters indicate significant differences between control (CS) and treatment (ANOVA; $p < 0.05$). Bars indicate SE.

Dunnet's test; Table 2). The total duration from the 1st to 4th instars was significantly longer in the Betzes treatment than in the CS control ($p < 0.01$; Dunnet's test; Table 2). Although the differences were statistically insignificant, the nymphs fed with 2H tended to take longer to grow during the first 2 instars than those fed with CS (Table 2).

In solitarious nymphs, which were derived from adults reared in isolation, the percentage of individuals that consumed leaves was also lower in the Betzes treatment than in the CS control (chi square test; $p < 0.01$; Table 3). Nymphal survival rates during the 1st, 3rd, 4th and 5th instars were significantly lower in the 5H and Betzes treatments than in the CS control (chi square test; $p < 0.01$; Table 3). The

Table 1 | Percentages of *Locusta migratoria* nymphs (gregarious phase, Minamidaito strain) that consumed leaves and survival rates of nymphs that were reared in isolation and fed with one of barley chromosome addition lines of wheat (2H–7H), barley cultivar 'Betzes' (B) or wheat cultivar 'Chinese Spring' (CS, control) until ecdysis to the 5th nymphal instar

	n	feeding[a] (%)	2nd instar (%)	3rd instar (%)	4th instar (%)	5th instar (%)
2H	26	22 (84.6)	19 (73.0)	15 (57.6)	14 (53.8)	13 (50.0)
3H	26	21 (80.7)	19 (73.0)	15 (57.6)	13 (50.0)	13 (50.0)
4H	26	23 (88.4)	20 (76.9)	14 (53.8)	13 (50.0)	11 (42.3)
5H	26	25 (96.1)	23 (88.4)	16 (61.5)	16 (61.5)	13 (50.0)
6H	26	25 (96.1)	21 (80.7)	15 (57.6)	14 (53.8)	10 (38.4)
7H	26	24 (92.3)	20 (76.9)	18 (69.2)	15 (57.6)	13 (50.0)
B	24	11 (45.8)**	4 (16.6)***	4 (16.6)***	4 (16.6)**	4 (16.6)**
CS	25	22 (88.0)	20 (80.0)	17 (68.0)	16 (64.0)	16 (64.0)

Asterisks indicate significant differences in percentage of hatchlings consumed leaves and survival rates from the 1st to respective instars between nymphs fed wheat and barley (chi square test; $^{**}p < 0.01$; $^{***}p < 0.001$).
[a]The number of hatchlings that consumed leaves.

Table 2 | The time (days ± SE) spent at each instar for *Locusta migratoria* nymphs (gregarious phase, Minamidaito strain) that were reared in isolation and fed with one of barley chromosome addition lines of wheat (2H–7H), barley cultivar 'Betzes' (B) or wheat cultivar 'Chinese Spring' (CS, control) until ecdysis to the 5th nymphal instar. The number of individuals examined was shown in Table 1

	1st instar	2nd instar	3rd instar	4th instar	1st–4th instars total
2H	10.9 ± 0.3	10.9 ± 0.5	9.7 ± 0.5	10.7 ± 0.5	39.2 ± 0.7
3H	9.2 ± 0.3	8.3 ± 0.5	9.0 ± 0.5	11.7 ± 0.5	36.4 ± 1.8
4H	10.0 ± 0.3	8.7 ± 0.5	9.1 ± 0.5	11.6 ± 0.5	37.3 ± 1.2
5H	9.6 ± 0.3	9.4 ± 0.5	9.4 ± 0.4	10.8 ± 0.5	37.4 ± 1.0
6H	9.5 ± 0.3	8.5 ± 0.5	8.8 ± 0.5	11.0 ± 0.5	36.6 ± 1.0
7H	9.9 ± 0.3	8.8 ± 0.4	8.8 ± 0.4	11.8 ± 0.4	37.8 ± 0.8
B	13.4*** ± 0.7	11.3 ± 0.9	8.5 ± 0.8	12.5 ± 0.8	45.7** ± 0.7
CS	9.4 ± 0.3	8.9 ± 0.5	8.6 ± 0.4	11.4 ± 0.4	37.7 ± 0.6

Asterisks indicate significant difference from the control (Dunnet's test; $^{**}p < 0.01$; $^{***}p < 0.001$).

duration of the 1st instar was longer in the Betzes treatment than in the CS control ($p < 0.001$; Dunnet's test; Table 4). Nymphs fed with 2H took significantly longer during the 1st and 2nd instars than did the control nymphs ($p < 0.01$ for 1st instar; $p < 0.05$ for 2nd instar; Dunnet's test; Table 4). The total duration from the 1st to 4th instars was significantly longer in the Betzes treatment than in the CS control ($p < 0.01$; Dunnet's test; Table 2), while it was significantly shorter in the 6H treatment versus the CS control($p < 0.001$; Dunnet's test; Table 2).

Discussion

A previous study revealed that locomotor activity of *L. migratoria* nymphs become very high when they are deprived of food but the activity declines after they are provided with food[18]. In the present study, locomotor activity of *L. migratoria* nymphs kept with barley or filter paper in the actograph was very high at the beginning and gradually decreased as the time elapsed (Figs. 1 and 2). In contrast, nymphs kept with wheat (CS control) had decreased locomotor activity during the first 6 hours or so during which nymphs probably settled and started feeding. The nymphs were placed in the actograph after 24 hours of hatching and thus should have started feeding as soon as they were introduced to the actograph[18]. Their locomotor activity remained at a very low level from 6 to 10 hours when the nymphs were probably actively feeding or resting. They became active again after this period, probably because they finished all the supplied leaves. The relatively high levels of locomotor activity and subsequent gradual decline shown by the nymphs kept with barley or filter paper might have been caused by the absence of food and subsequent exhaustion. It is possible that barley leaves were not recognized as food by *L. migratoria* hatchlings. On the other hand, the level and pattern of locomotor activity for the nymphs kept with 2H–7H were similar to those for the nymphs kept with CS, suggesting that *L. migratoria* may recognize the wheat-barley chromosome addition lines as food.

The amount of leaves consumed by locusts varied depending on which barely chromosome was added to the wheat. In the no-choice feeding test, the amount of leaves consumed within 24 hours was lower in 5H and 6H than in the other chromosome addition lines and CS (Fig. 3). In the choice feeding test, the amount of leaves consumed within 44 hours was lower in 2H, 5H and 6H than in CS (Fig. 4). These results suggest that the barley genes discouraging continuous feeding of the migratory locust are located on barley chromosomes 5H and 6H and those influencing the attractiveness of chromosome-added wheat as food to the locust on barley chromosomes 2H, 5H and 6H. These results strongly suggest that barley metabolites other than gramine are associated with the feeding deterrence because of the following reasons. Gramine contents greatly vary with barley cultivars[19–21] and Betzes is known as a gramine-free cultivar[22]. In addition, the gene coding *N*-methyltransferase, a key enzyme related to gramine biosynthesis, is located on chromosome 1H (previously designated as chromosome 5)[21,23–25], of which addition line was unavailable in this study.

Although a previous study reported that adults of *L. migratoria* did not eat barley seedlings[12], in our study nearly half of hatchlings consumed Betzes leaves (Tables 1 and 3). This difference may suggest that the amount of feeding deterrent (e.g. gramine and other metabolites) present in Betzes leaves is smaller than in the barley cultivars (cv. 'Aizu-6' etc.) used by the previous study[12]. Alternatively, the *L. migratoria* strain used by the previous studies[12,13], an uncertain origin; supplied by the Insectarium, Tama Metropolitan Zoo, Japan, might have been more susceptible to barley than the strains we used in this study. Further studies are needed to clarify the factors responsible for the above difference observed between the two studies.

Table 3 | Percentage of *Locusta migratoria* nymphs (solitarious phase, Minamidaito strain) that consumed leaves and survival rate of nymphs that were reared in isolation and fed with one of barley chromosome addition lines of wheat (2H–7H), barley cultivar 'Betzes' (B) or wheat cultivar 'Chinese Spring' (CS, control) until ecdysis to the 5th nymphal instar

	n	feeding[a] (%)	2nd instar (%)	3rd instar (%)	4th instar (%)	5th instar (%)
2H	31	24 (77.4)	19 (61.2)	17 (54.8)	17 (54.8)	17 (54.8)
3H	26	22 (84.6)	17 (65.3)	16 (61.5)	16 (61.5)	15 (57.6)
4H	27	26 (96.2)	19 (70.3)	13 (48.1)	13 (48.1)	13 (48.1)
5H	27	24 (88.8)	17 (62.9)	11 (40.7)**	10 (37.0)**	10 (37.0)**
6H	27	24 (88.8)	19 (70.3)	18 (66.6)	18 (66.6)	18 (66.6)
7H	27	26 (96.2)	20 (74.0)	16 (59.2)	16 (59.2)	16 (59.2)
B	31	18 (58.0)**	16 (51.6)	12 (38.7)**	11 (35.4)**	10 (32.2)**
CS	32	30 (93.7)	25 (78.1)	24 (75.0)	24 (75.0)	24 (75.0)

Asterisks indicate significant differences in percentage of hatchlings that consumed leaves and survival rates from the 1st to respective instars between nymphs fed with wheat and barley (chi square test; $^{**}p < 0.01$; $^{***}p < 0.001$).
[a]The number of hatchlings that consumed leaves.

Table 4 | The time (days ± SE) spent at each instar (days ± SE) of *Locusta migratoria* nymphs (solitarious phase, Minamidaito strain) that were reared in isolation and fed with one of barley chromosome addition lines of wheat (2H–7H), barley cultivar 'Betzes' (B) or wheat cultivar 'Chinese Spring' (CS, control) until ecdysis to the 5th nymphal instar. The number of individuals examined is shown in Table 3

	1st instar	2nd instar	3rd instar	4th instar	1st–4th instars total
2H	10.8** ± 0.5	10.2* ± 0.5	9.4 ± 0.4	11.0 ± 0.4	41.2 ± 0.9
3H	9.0 ± 0.5	9.7 ± 0.5	7.8 ± 0.4	9.7 ± 0.5	36.1 ± 1.0
4H	10.3 ± 0.5	9.6 ± 0.5	8.4 ± 0.5	10.5 ± 0.5	38.8 ± 0.7
5H	10.4 ± 0.5	9.0 ± 0.6	8.0 ± 0.5	8.6 ± 0.6	36.1 ± 0.6
6H	9.0 ± 0.5	8.2 ± 0.4	7.6 ± 0.4	9.4 ± 0.4	33.8*** ± 0.6
7H	9.0 ± 0.4	8.6 ± 0.5	8.4 ± 0.4	10.8 ± 0.4	36.8 ± 0.8
B	12.8*** ± 0.5	11.0 ± 0.5	9.9 ± 0.5	10.7 ± 0.6	44.1** ± 2.7
CS	10.1 ± 0.4	9.3 ± 0.4	8.6 ± 0.3	10.4 ± 0.4	38.5 ± 0.8

Asterisks indicate significant differences from the control (Dunnet's test; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Developmental performance evaluated by nymphal survival and growth was reduced when the nymphs were provided with Betzes instead of wheat both in gregarious and solitarious hatchlings (Tables 1–4). No significant difference was detected between wheat-barley chromosome addition lines and CS control in either survival rate or nymphal development for gregarious nymphs (Tables 1 and 2). In solitarious nymphs, however, the corresponding differences were statistically significant (Tables 3 and 4). The survival rate for the solitarious nymphs became lower in those fed with 5H than in those fed with CS (Table 3). The duration of the 1st instar was prolonged when the hatchlings were fed with 2H compared with those fed with CS. These results suggest that the barley genes influencing the survival rate and nymphal development of *L. migratoria* exist on barley chromosomes 5H and 2H, respectively, and the effects of these genes were phase-dependent in the locust.

Because all test nymphs were individually reared, the differences observed in survival rate and nymphal development between the gregarious and solitarious nymphs are likely caused by the parental rearing density or phase. It is possible that gregarious hatchlings that are likely to face a food shortage under crowded conditions are more tolerant of suboptimal food conditions than solitarious hatchlings that normally occur at low population densities.

As has been suggested by a previous research[13], feeding deterrence of barley against *L. migratoria* is probably caused by multiple factors. Results of our study support their consideration. As mentioned above, our study suggested that the barley gene(s) related to inhibition of feeding by *L. migratoria* are located on barley chromosomes 5H and 6H, those related to the palatability of plants on chromosomes 2H, 5H and 6H, and those negatively affecting the survival and nymphal growth of the locust on chromosomes 5H and 2H.

Although an available gene database of barley consists fundamentally of genes involved in morphological phenotypes alone[26], recent progress in barley genome study[27] will enable us in the near future further detections of candidate barley genes related to feeding behavior and developmental performance of *L. migratoria*, which are definitely useful for developing novel resistant varieties of poaceous crops against the migratory locust.

Methods

Laboratory-reared strains of *Locusta migratoria*. Two laboratory-reared strains of *L. migratoria*, originated from Iheya and Minamidaito Islands, Okinawa, Japan were used in the experiments. The Iheya strain was originally derived from Iheya Island in October 2006 and has been reared in the locust laboratory, NIASO, Tsukuba, Japan. The Minamidaito strain originally collected on Minamidaito Island in February 2007 has been reared in the same laboratory. These strains are genetically very close to each other[6].

Eggs of solitarous phase locusts were obtained by rearing female adults of *L. migratoria* individually in small cages (28 cm long, 15 cm wide and 28 cm high) except for a few days for mating with a male at 30 ± 1°C under an LD 16 : 8-h photocycle. Eggs of gregarious phase locusts were obtained by rearing adults in groups of approximately 100 individuals in large cages (42 cm long, 22 cm wide, 42 cm high) under the same environmental conditions. Fresh leaves of Eulalia grass *Miscanthus sinensis* Anderss. (Poaceae), sorghum, or rescue grass *Bromus catharticus* Vahl. (Poaceae), depending on the season, and a small amount of wheat bran were provided as food[28].

For oviposition, a small plastic cup (9.0 cm diameter and 4.0 cm deep) filled with moist sand was put in each cage. Cups were checked for oviposition every day and those containing egg pods were stored at 20°C temporarily in an incubator and transferred to another incubator at 30°C prior to experiments for promoting egg hatching.

Wheat-barley chromosome addition lines. As test plants, *T. aestivum* cv. Chinese Spring, *H. vulagare* cv. Betzes, and six Betzes chromosome disomic addition lines (2H–7H) of CS, obtained from the National Bioresource Project-Wheat, Japan, were used in this study. These chromosome addition lines have been developed through wide hybridization between hexaploid wheat (CS) and diploid barley (Betzes). Each line contains the full set of wheat chromosomes and a single chromosome pair from barley.

Locomotor activity in *L. migratoria* nymphs exposed to wheat, barley and barley chromosome addition lines of wheat. Locomotor activity of nymphs was quantified with an actograph system[18,29] at 30 ± 0.5°C under continuous light conditions. To examine behavioral responses to wheat and barley, gregarious phase nymphs of the Iheya strain that were fasted for 24 hours after hatching were individually placed in transparent cylindrical containers (18 mm diameter and 74 mm long) with a piece (ca. 20 mg) of fresh leaf of CS, Betzes, or moist filter paper (negative control) and subjected to the actograph with an infrared motion sensor. Locomotor activity was evaluated by the number of times nymphs crossed the infrared beam per hour. This experiment was continued for 18 hours and the numbers of replications were 33, 27 and 31 for Betzes, CS and filter paper, respectively.

Then locomotor activity in *L. migratoria* nymphs kept with various barley chromosome addition lines was surveyed. Gregarious phase nymphs of the Iheya strain that have been fasted for 24 hours after hatching were individually placed in the transparent cylindrical containers with a piece (ca. 20 mg) of fresh leaf of CS, Betzes, or 2H–7H and subjected to the actograph with an infrared motion sensor as described above. Locomotor activity was evaluated as described above. This experiment was continued for 16 hours and the numbers of replications were 12, 13, 14, 13, 14, 13, 12 and 14 for Betzes, CS, 2H, 3H, 4H, 5H, 6H and 7H, respectively.

Effects of barley chromosome addition on the acceptability of wheat by *L. migratoria* (no-choice test). To examine the effects of barley chromosome addition on the acceptability of wheat by *L. migratoria*, gregarious locust hatchlings of the Iheya strain that had been fasted for 24 hours since hatching were individually put into petri dishes (9.0 cm diameter and 2.0 cm deep), each containing 100 mg of fresh first leaves of CS, Betzes, or one of 2H–7H. The basal tips of leaves were put into 0.2 ml microtubes filled with tap water to avoid wilting. The amount of leaves consumed by each locust was determined 1, 4, and 24 hours later by weighing the remaining leaves. Six replications were conducted for respective treatments.

Effects of barley chromosome addition to wheat on the preference of *L. migratoria* (choice test). To examine the effects of barley chromosome addition to wheat on the feeding preference of *L. migratoria*, gregarious hatchlings of the Iheya strain that had been deprived of food for 24 hours since hatching were individually put into petri dishes (9.0 cm diameter and 2.0 cm deep), each containing 100 mg of fresh first leaves of CS and one of 2H–7H as prepared as above. The amount of leaves consumed was determined by weighing the remaining leaves of the two plant types separately 4, 8, 20, and 44 hours later. Six replications were conducted for respective treatments.

Effects of barley chromosome addition to wheat on the survival and development of *L. migratoria*. To examine the effects of barley chromosome addition to wheat on the survival and development of *L. migratoria*, locust nymphs were reared with CS, Betzes or one of 2H–7H.

Newly hatched nymphs were reared in isolation in petri dishes (9.0 cm diameter and 2.0 cm height) at 25°C under an LD 16 : 8-h photocycle and were transferred into plastic containers (12 cm long, 12 cm wide and 5.5 cm height) upon ecdysis to the

2nd instar. Nymphs were fed CS, Betzes or one of 2H–7H until they attained the 5th instar or died. Nymphs were observed daily and the survival rate and duration of each nymphal instar (first to fourth) were examined. At the same time, whether hatchlings consumed the leaves or not was observed during the first instar by checking the leaves of test plant visually. The number of individuals examined was shown in Tables 1 and 3.

Statistical analyses. Locomotor activity of *L. migratoria* hatchlings was analyzed by ANOVA followed by Tukey's HSD test for those kept with CS, Betzes, or moist filter paper or by Dunnet's test between the control (CS) and treatments for the experiment using 2H–7H. The amount of leaves consumed by hatchlings at each time after the start of the no-choice feeding test was analyzed using Dunnet's test between the control (CS) and respective treatments. The amount of leaves consumed by hatchlings in the choice test was analyzed by ANOVA for each treatment. Nymphal survival rate and growth period were analyzed by chi square test and Dunnet's test, respectively, between the control (CS) and respective treatments.

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Author contributions

M.T., S.T., K.K., Y.O., Y.W. and O.S. conceived and designed the study. S.S., K.H., S.T. and M.T. performed the experiments. S.S., K.H. and M.T. analyzed data. M.T., S.S. and S.T. wrote the paper.

Additional information

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