The impact of selection on population genetic structure in the clam *Meretrix petechialis* revealed by microsatellite markers

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Received: 20 January 2015 / Accepted: 13 November 2015 / Published online: 19 November 2015
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Abstract The aim of our work is to evaluate the impact of mass selection on genetic structure in artificially closed populations of the clam *Meretrix petechialis*. In the present study, we performed mass selection over four generations (from 2004 to 2010) on two clam populations [shell features of purple lines (SP) and black dots (SB)] and analyzed their temporal genetic variation and structure using microsatellite makers. The two closed populations originated from the natural Shandong population (SD); thus, a natural SD population (10SD) was used to detect the current genetic structure after 6 years of natural selection. The results showed that the genetic diversity of the four generations of SB and SP was gradually reduced but remained at relatively high levels (SB, \(A = 18.94–16.8\), \(H_o = 0.7389–0.6971\), and \(H_e = 0.8897–0.8591\); SP, \(A = 20.0–17.8\), \(H_o = 0.7512–0.7043\), and \(H_e = 0.8938–0.8625\)), which has not been reduced compared with that of the 10SD population (\(A = 17.8\), \(H_o = 0.6803\), and \(H_e = 0.8302\)). The \(N_e\) estimates for the two populations were almost at the same levels as the actual numbers of parental individuals. In addition, a low inbreeding coefficient was detected in the two populations (SB, 0.00201–0.00639; SP, 0.00176–0.00541). Based on the results, the present mass selection has not made a large impact on the population genetic structure of the closed populations. The present investigation provides important information for the development of management strategies for genetic breeding of the clam.

Keywords Mass selection · *Meretrix petechialis* · Microsatellite · Population genetic structure · Temporal genetic variation

Introduction

The clam *Meretrix petechialis* (also incorrectly described as *M. meretrix* in some papers) (Chen et al. 2011; Zhang et al. 2012) is one of the important commercial aquaculture bivalves in the coastal areas of Asia (Tang et al. 2006). In past decades, the seeds for clam aquaculture in China were mainly provided by growth-advantaged natural populations, e.g., the natural Shandong population (SD) (Liu et al. 2006). However, in recent years, the natural clam resources have declined dramatically, and their genetic diversity is fragile as a result of habitat destruction, climate changes, environmental pollution and over-fishing (Crozier 2000; Bakos and Gorda 2001; Cross 2001). Many marine organisms such as shrimp and fish suffer from the same situation (Worm et al. 2006; Gillett 2008; Davies et al. 2009). As an alternative, genetic breeding programs have been developed for *M. petechialis*. In such programs, a batch of animals is initially selected from a natural population as broodstock for hatchery. Subsequently, their offspring, having traits of economic importance (such as growth and disease resistance), were selected as parents for the production of new generations. During this successive selection process, no genetic material was introduced to the...
breeding population, which might reduce the genetic diversity of the closed populations by selective pressure and inbreeding (Nielsen 1998; Shaklee et al. 1999). However, in most cases, little attention has been paid to the degree of such an impact on the selected populations. This uncertainty could prevent the achievement of desired gains and long-term sustainability of the breeding program.

As some economic traits (such as growth performance) are positively related to heterozygosity (Thiriot-Quèvreux et al. 2011; Lu et al. 2012) and genetic diversity is related to the sustainability of populations (Ellstrand and Elam 1993; Lande and Shannon 1996; Landergott et al. 2001), it is essential to investigate the impact of mass selection on genetic diversity of artificially closed populations for further aquaculture production. Because of the limitations of current statistical methods, the analysis of those genetic parameters (such as Ho and He) was probably insufficient to achieve accurate genetic information of the populations (Consuegra et al. 2005). The effective size of a population (Ne) has been widely used to determine the levels of genetic diversity of a population (Montgomery et al. 2000; Bollback et al. 2008). Ne can indicate the loss rate of genetic diversity, the fixation rate of deleterious alleles, and the impact of selection on the maintenance of beneficial alleles (Wang 2001; Kalinowski and Waples 2002). In addition, Ne is a key factor in efforts of genetic diversity conservation and breeding management (Waples 1990; Berthier et al. 2002; Kalinowski and Waples 2002; Shrimpton and Heath 2003). Previous studies have reported that a small Ne in cultured stocks could reduce genetic diversity through a combination of genetic drift and inbreeding (Hedrick and Kalinowski 2000; Frankham et al. 2002; Frankham 2005; Gauffre et al. 2008). Therefore, it is important to estimate Ne in cultured populations (Waples 1989a; Beaumont 1999; Bollback et al. 2008). The standard temporal method (Waples 1989a) is by far the most widely used approach for estimating Ne (Waples and Yokota 2007), which is based on the relationship between Ne and the standardized variance of change on allele frequency among different generations or samples taken at different times (Waples 1989a). In recent years, two new methods for Ne estimation, Pseudo Maximum Likelihood method (Wang and Whitlock 2003) and Maximum Likelihood-based estimation (Berthier et al. 2002), have been proposed to obtain information about the full distribution of allele frequency and to reduce bias.

In this study, we performed mass selection over four generations (from 2004 to 2010) on two clam populations to improve their growth traits, during which no natural resources were introduced into the two populations. Microsatellite markers were used to analyze temporal variation of genetic diversity in the generations of the two closed populations, as microsatellites have been successfully applied for genetic monitoring (Greg et al. 1997; Lemus-Flores et al. 2001; Gour et al. 2006; Li et al. 2006; Lin et al. 2008). In our previous studies, the genetic diversity of the clam populations was low (Lu et al. 2012), and it declined largely after selection for one generation with the selection intensity of the top 15 % (Lu et al. 2013). To improve growth performance and avoid fast decrease of genetic diversity in the present initiatory selection breeding program, we performed a relatively lower selection intensity (top 30 %) in initial generations of the present two nucleus populations. Under the present selection intensity, the growth performance of the selected populations increased about 20 % after four generations, but the degree of impact on genetic diversity was unclear. The objective of this study was to evaluate the impact of the present intensity of mass selection on population genetic diversity in the clam populations. The results will provide insight for improving management strategies for the genetic breeding of M. petechialis and other marine bivalves.

Materials and methods

Selection process of the clam M. petechialis

In our selective breeding program, four generations of two M. petechialis populations were produced from 2004 to 2010 in the hatchery laboratory of the Zhejiang Mariculture Research Institute (Wenzhou, Zhejiang, China). In 2004, two base populations were constructed by 500 matured clams with an approximate sex ratio of 1:1 that were chosen based on shell features of purple lines (SP) and black dots (SB) from a natural Shandong population (SD) in Dongying, Shandong, China. The shell color features (SP and SB) were used for stock differentiation in the subsequent selection process (Fig. 1). The first generations (04G 1SB and 04G 1SP) were produced using the two base populations. In June 2006, when the individuals of 04G 1SB and 04G 1SP were mature, 500 clams, the upper one third of individuals in each closed population, were selected to produce the second generations of 06G 2SB and 06G 2SP. Similarly, the third generations of 08G 3SB and 08G 3SP, and the fourth generations of 10G 4SB and 10G 4SP were subsequently obtained in 2008 and 2010, respectively.

Adult conditioning and larval rearing followed the method described by Liu et al. (2006). Briefly, the selected parental clams were conditioned in seawater from 20 to 26 °C for over 10 days, and they were fed with mixed algal diet of Chaetoceros muelleri and Dicrateria inornata. For each population, all of the parental clams were mixed together and induced to spawn by a combination of exposure to air in the dark for 4–6 h and temperature shock (28 °C) in filtered sea water, and approximately 60 % of
the clams of each population were induced to spawn to produce offspring. After spawning, fertilized eggs of each population were incubated in a separate tank filled with filtered seawater until they developed into the D-larvae stage after 18 h at 28 °C, and then the fertilized eggs were reared at the density of 5 individuals per ml. When most of the larvae had settled (pelagic larvae accounted for less than 5% in the cohorts), a 0.5 cm layer of sand was placed on the bottom of the tanks for their settlement. The seawater was replaced once a day with water temperature kept at 28 ± 1 °C at larval stage or 27 ± 1 °C for juveniles, and the salinity was maintained at 23.0 ± 1%. The animals were fed with D. inornata three times a day at the larval stage, and juveniles were given a mixed diet of C. muelleri and D. inornata. When the average shell length of juvenile clams reached 2.5 mm, the clams were deployed to cement tanks, and when the shell length of the juvenile clams reached 4–5 mm, the clams were transferred to outdoor ponds for continued growth. The experimental sampling was conducted when the clams of the second, third, and fourth generations reached one year of age. The wild SD individuals were collected from the same locality as those of the two founding populations in 2010 (10SD) to investigate the present genetic diversity of the natural population. A total of 396 clams were randomly sampled in this study (Table 2). All the sampled clams were stored in 95% ethanol for subsequent genetic analyses.

DNA extraction and microsatellite genotyping

For genotyping, total genomic DNA was extracted from clam muscle tissues using TianGen Tissue Sample Kit (TianGen, Beijing, China) and stored at −20 °C (Taggart et al. 1992). Nineteen microsatellite loci with high polymorphism were used for genetic analysis in this study, including MM09, MM11, MM14, MM15, MM16 (Lu et al. 2011), MM12295, MM5358, MM6277, MM6863, MM8105, MM3923, MM6717, MM1272, MM8365, MM12736, MM26715 and MM2016 (Lu et al. 2011), and two new loci, MM1031 and MM21723 (Table 1). PCR was conducted in a 10 μl reaction mixture containing 1× PCR buffer (Promega, USA), 1.25 μmol of MgCl₂, 50 μmol of each dNTP, 0.25 mmol of each primer, 0.5 U of Taq DNA polymerase (Promega), and 10 ng of template DNA. On a MJ PCR-200 thermal cycler (Bio-Rad, USA), the thermal cycling condition were initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, anneal at annealing temperature for 30 s (Lu et al. 2011; 2012; Table 1), and extension at 72 °C for 45 s with an additional 10 min at the end. The size of the alleles was determined using 8% non-denaturing acrylamide gels with a 50 bp DNA Ladder as a standard DNA marker (Promega, USA). Each individual diploid genotype was quantified using Quantity-One software (v 4.5, Bio-Rad).

Analysis of genetic diversity of the closed populations and wild population

To check for potential genotyping errors, all of the genotype data were tested with Micro-checker software (Van Oosterhout et al. 2004). The number of alleles at each microsatellite locus (N_a), the proportion of individual samples that were heterozygous (direct count heterozygosity, H_o) and the unbiased estimate of heterozygosity (H_e) for each generation of the two closed populations were assessed using Cervus version 3.0 (Kalinowski et al. 2007). Allelic richness (A) was determined using the FSTAT 2.9.3 program (Goudet 1995, 2001; Leberg 2002) to avoid derived bias in a comparison of the mean number of alleles (N_a), H_o and H_e. In addition, a difference in the genetic diversity between samples was detected using a t test as a paired observation. Deviation from Hardy–Weinberg Equilibrium (HWE), linkage disequilibrium and differences in allele frequencies among samples were all tested using GENEPOP version 4.0.10 (Raymond and Rousset 1995; available at http://genepop.curtin.edu.au).
Analysis of genetic differentiation between contemporary or temporal samples

Among the contemporary samples of the same generation of different populations, the population differentiation was assessed using the FSTAT statistical package (2.9.3 Version) (Rice 1989; Goudet 2001). However, to detect the discrepancy among generations in each population, a modified statistic F_T analogue to F_ST was used, as suggested by Sandoval-Castellanos (2010). The F_T was calculated as F_T = F_ST − F^S, where F^S was the mean of gene drift, which was the average of F_ST among successive generations. F_ST was calculated using a formula of F_ST = (H^T − H^S)/H^T, where H^S was the average Hardy–Weinberg heterozygosity, and H^T was derived from a formula of H^T = 1 − \sum p_i^2 with any number of alleles p_i (Nei 1973).

Estimation of the effective population size (Ne) of the closed populations and wild population

Based on allele frequency changes, the effective population size (Ne) of the generations was estimated using Temporal Methods (Pollak 1983; Waples 1989b, 1990; Sandoval-Castellanos 2010). Briefly, the adjusted F values (F_a) of the sample size were used to compare allele frequency variances between samples, which were calculated using a formula of F_a = F − 1/S (S was the harmonic mean of sample sizes in two populations). Then, Ne was calculated as Ne ≈ t/[2(F − 1/S)], where F was the standardized variance of the allele frequency change, and t was the number of generations between the samples. Ne of the generations was also estimated using two different likelihood-based temporal methods with the software programs TM3.1 (Berthier et al. 2002) and MLNE (Wang and Whitlock 2003). As suggested by Berthier et al. (2002), the maximum estimation (500–10,000) of Ne can be varied with 20,000–50,000 of iterations. Finally, the 95 % confidence intervals (CIs) were calculated for Ne estimation using \chi^2 approximation (Waples 1989b).

Detection of inbreeding evaluation

As the rate of inbreeding was dependent on the effective population size (Ne) (Falconer 1989), an inbreeding coefficient (F) was calculated using the equation of F = ΔF + (1 − ΔF)/F_{t−1}, where ΔF = 1/(2Ne). In closed populations, the first generations of 04G_{SP} and 04G_{SB} were collected from a natural Shandong population. Because we have not collected natural samples at the beginning of the breeding program, the F_{in} value of the 10SD population and its inbreeding coefficient had to be used as F_{1} for the calculation of F_2 in SP and SB populations, although that value might be an overestimation.

Results

Description of contemporary or temporal variability in genetic diversity

Analysis with the Micro-checker software revealed that null alleles were likely present in 11 loci. To avoid bias derived from those null alleles, the remaining 8 loci were used for genetic analyses (Table 1). The 8 microsatellite loci showed a high level of polymorphism, with the mean alleles (Na) ranging from 17.8 to 22.5 among the samples (Table 2). After Bonferroni correction, no deviation from Hardy–Weinberg Equilibrium (HWE) was detected at any of the loci, and no linkage disequilibrium was detected among the eight microsatellite loci.

The contemporary samples of 10G_{SB}, 10G_{SP} and 10SD has similar genetic diversity (Table 2), which had mean Ho values of 0.6971, 0.7074, and 0.6803.
Table 2 Analysis of genetic diversity in different populations of M. petechialis

| Population | N  | Na | Ho   | He  | A   | F   |
|------------|----|----|------|-----|-----|-----|
| 06G4SP     | 50 | 22.5 | 0.7512 | 0.8938 | 20.0 | 0.00176 |
| 08G4SP     | 64 | 21.4 | 0.7264 | 0.8716 | 19.4 | 0.00349 |
| 10G4SP     | 64 | 19.8 | 0.7043 | 0.8625 | 17.8 | 0.00541 |
| 06G4SB     | 50 | 20.7 | 0.7389 | 0.8897 | 18.9 | 0.00201 |
| 08G4SB     | 64 | 19.3 | 0.7193 | 0.8642 | 18.1 | 0.00410 |
| 10G4SB     | 64 | 18.5 | 0.6971 | 0.8591 | 16.8 | 0.00639 |
| 10SD       | 40 | 17.8 | 0.6803 | 0.8302 | 15.9 | 0.000102 |

N, number of studied individuals; Na, mean number of alleles; Ho, mean observed heterozygosity; He, mean expected heterozygosity; A, mean allele richness; F, inbreeding coefficient; SP, population of shell purple lines; SB, population of shell black dots; SD, wild Shandong population; G2, the second generation; G3, the third generation; G4, the fourth generation

Regarding genetic differentiation and inbreeding coefficient (F) in different generations of each population, the mean values were higher in the second generation (F = 0.153; 10G4SP and 10SD, F = 0.168). Furthermore, neither SB nor SP showed a low degree of temporal differentiation between generations (Table 3).

Allele frequency distribution varied substantially among generations of both SP and SB. The highest values of adjusted standardized variance of allele frequency change (Fa) were obtained between 06G4SB and 10G4SB (0.00419) in SB and between 06G4SP and 10G4SP (0.00347) in SP. The remaining Fa value is very close to those in both SB (06G2SB–08G4SB, 0.00191; 08G4SB–10G4SB, 0.00231) and SP (06G2SP–08G4SP, 0.00166; 08G4SP–10G4SP, 0.00192) (Table 4).

Temporal changes in the effective population size (Ne)

For each generation in both SB and SP, the Ne estimates from the 3 methods were not changed significantly in this study (Table 5), thus we used the medium data from the standard temporal method of TMW for analysis. The Ne ranged from 262.0 to 216.7 in SB, and from 301.5 to 260.1 in SP (Table 5). For the comparison in each population, the lowest Ne value was obtained in the fourth generation (10G4SP, Ne = 260.1; 10G4SB, Ne = 216.7), whereas the highest value was obtained in the second generation (06G2SP, Ne = 301.5; 06G2SB, Ne = 262.0).

Inbreeding detection

The mass selection process of the breeding program, resulted in an inbreeding coefficient (F) in the range of 0.00201 to 0.00639 in the SB generations, and a range of 0.00176 to 0.00541 in SP. The inbreeding coefficient was 0.000102 in the 10SD population (Table 2).

Discussion

According to previous reports, successive closed breeding with a limited number of parental founders could lead to a reduction in genetic diversity and the effective population size, which could increase the rate of both inbreeding and genetic drift (Falconer 1989; Wang and Ryman 2001; Hartl and Clark 1997; Lu et al. 2010); in a small population the probability of mating with a relative and losing alleles by chance are increased (Ellstrand and Elam 1993; Li et al. 2004). In the present investigation, eight microsatellites were used for genetic analysis in two closed clam populations (SP and SB) that were selected for four generations by mass selection. The mass selection was conducted in a batch with limited clams and no genetic materials were introduced. The results showed that the observed heterozygosity of the SP and SB populations was reduced...
6.24 and 5.66 %, respectively, after the selection of several successive generations. However, in our previous study, the observed heterozygosity reduced 11.48 % after the selection of one generation with the selection intensity of the top 15 % (Lu et al. 2013). The relatively small decrease in genetic variation in the two populations over four generations might mainly result from the lower selection intensity (top 30 %) in the present breeding program. In addition to the impact from closed breeding with mass selection, there may be some other factors that contribute to the reduction in the values of heterozygosity and allele richness over generations in both SB and SP, such as the differences in juvenile survival and the proportion of males and females involved in spawning. Lu et al. (2010) have reported that the population size, sex ratio and the initial frequency of alleles were important factors affecting the genetic variation of closed populations. However, in many aquaculture species, especially in clam, it is difficult to control the sex ratio of parents, as the spawning and mating is random in closed populations. In addition to genetic diversity, no significant genetic differentiation was detected between the two populations. Compared to the 10SD population, the mean values of expected heterozygosity and allele richness in the two populations were all at a high level, which indicated that the genetic diversity of the closed breeding populations was not seriously affected by mass selection.

However, notably, important biological differences cannot always be fully detected using statistical analysis (Waples 1989a). As addressed in the study of Atlantic salmon populations by Consuegra et al. (2005), the general analysis of those genetic parameters (such as \( H_o \) and \( H_e \)) was probably not sufficient to achieve accurate genetic information for those populations. As an alternative, \( N_e \) estimates in this study could provide more substantial information for genetic monitoring of the clam populations. When the three methods were applied for the temporal analysis, \( N_e \) estimates for the generations of SB or SP were not evidently different. If the clams that were induced to spawn contributed equally per generation and without selection, the \( N_e \) estimates might be maintained at the same level over generations for each population. However, \( N_e \) estimates were going through a

| Table 3 | \( F_t \) estimation of temporal genetic differentiation during the closed breeding of M. petechialis |
|---|---|
| 06G_{SB} – | 0.00042 | 0.00078 | 06G_{SP} – | 0.00029 | 0.00077 |
| 08G_{SB} – | 0.00036 | 0.00036 | 08G_{SP} – | 0.00023 | 0.00023 |
| 10G_{SB} – | 10G_{SP} – | 10G_{SP} – |

SP, population of shell purple lines; SB, population of shell black dots; G_{2}, the second generation; G_{3}, the third generation; G_{4}, the fourth generation

| Table 4 | Parameters for estimating effective population sizes over generations for two closed stocks and a wild Shandong population of M. petechialis |
|---|---|
| Population | F | Fa | t/b | S | IA |
| 06G_{SP}–08G_{SP} | 0.01947 | 0.00166 | 1 | 56.14 | 6 |
| 06G_{SP}–10G_{SP} | 0.02128 | 0.00347 | 2 | 56.14 | 15 |
| 08G_{SP}–10G_{SP} | 0.01755 | 0.00192 | 1 | 64.00 | 9 |
| 06G_{SP}–08G_{SP} | 0.01972 | 0.00191 | 1 | 56.14 | 7 |
| 06G_{SP}–10G_{SP} | 0.02200 | 0.00419 | 2 | 56.14 | 23 |
| 08G_{SP}–10G_{SP} | 0.01793 | 0.00231 | 1 | 64.00 | 12 |
| 10SD | 4 | 40 |

F, standardized variance of allele frequency change; Fa, sample size adjusted F; t/b, generation time between samples; S, pairwise harmonic mean sample size; IA, pairwise number of independent alleles; SP, population of shell purple lines; SB, population of shell black dots; SD, wild Shandong population; G_{2}, the second generation; G_{3}, the third generation; G_{4}, the fourth generation

| Table 5 | The effective population size (\( N_e \)) of M. petechialis during closed breeding with discrete generations and SD with overlapping generations |
|---|---|---|---|
| Ne (TMW) | Ne (MLNE) | Ne (TM3.1) |
| 06G_{SP} | 301.5 (204.3–429.6) | 332.5 (228.5–436.3) | 281.5 (185.6–379.8) |
| 08G_{SP} | 288.3 (195.4–412.3) | 309.5 (271.4–423.7) | 262.7 (161.2–364.3) |
| 10G_{SP} | 260.1 (163.1–397.7) | 284.7 (180.8–400.3) | 246.5 (152.7–348.3) |
| 06G_{SB} | 262.0 (198.1–337.9) | 275.9 (210.5–346.1) | 253.3 (182.3–320.2) |
| 08G_{SB} | 238.7 (186.5–321.4) | 268.2 (199.4–328.4) | 224.8 (175.9–310.6) |
| 10G_{SB} | 216.7 (189.1–317.3) | 257.4 (177.9–305.8) | 206.9 (165.2–303.7) |

Ne (TMW), effective population size estimates and their 95 % CIs using the method of Waples (1989a); Ne (MLNE), effective population size estimates and their 95 % CIs using the likelihood method of Wang and Whitlock (2003); Ne (TM3.1), effective population size estimates and their 95 % CIs using 20,000 iterations, by the likelihood method of Berthier et al. 2002; SP, population of shell purple lines; SB, population of shell black dots; G_{2}, the second generation; G_{3}, the third generation; G_{4}, the fourth generation
slight reduction over generations such that $06G_2$SP or $06G_2$SB had the highest values and $10G_4$SP or $10G_4$SB had the lowest values in SP and SB, respectively. Similarly, the relatively small decrease in effective population size in the present study might mainly result from the lower selection intensity. In addition to the effect from mass selection, the present decrease of $\text{Ne}$ over generations may be due to the different ratio of males and females induced to spawn, the different status of sexual maturity in parents, or random mating for the generations (Falconer 1989). However, these results suggested that a batch with limited founders (500 individuals) and random mating in closed breeding with mass selection could maintain genetic diversity for several generations.

As reported in animals and plants, inbreeding depression could occur for a selected trait if the trait has a linear decease with an increase of the inbreeding coefficient (Falconer 1989; Hauser and Loeschcke 1996; Analla et al. 1999; Shikano et al. 2001). Although the inbreeding coefficient in both SB and SP slightly increased over generations, the values were still at a relatively low level. This finding indicates that there is no need to extend the mating population to prevent inbreeding depression and to reduce the risk of genetic homogenization in the near future. However, further studies with more recent samples are needed for genetic monitoring in closed populations. Thus, with more accurate information, an appropriate management strategy can be conducted in a timely fashion.

In conclusion, although the two populations experienced a gradual decrease of genetic diversity over the four successive generations by mass selection, they did not suffer from significant genetic deterioration with the present selection intensity. In addition, series genetic monitoring of the closed breeding populations is recommended, although the current breeding approach has not caused genetic divergence. The present investigation provides important information for the development of appropriate management strategies for clam breeding.

Acknowledgments This project was financially supported by the Chinese National High-Tech R & D Program (2012AA10A410) and the Zhejiang science and technology project of agricultural breeding (2012C12907-4).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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