Development of markers using microsatellite loci of two rove beetle species, *Paederus fuscipes* Curtis and *Aleochara (Aleochara) curtula* Goeze (Coleoptera: Staphylinidae), followed by analyses of genetic diversity and population structure

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Abstract

**Background** The family Staphylinidae is the most speciose beetle group in the world. The outbreaks of two staphylinid species, *Paederus fuscipes* and *Aleochara (Aleochara) curtula*, were recently reported in South Korea. None of research about molecular markers and genetic diversity have been conducted in these two species.

**Objective** To develop microsatellite markers and analyze the genetic diversity and population structures of two rove beetle species.

**Methods** NGS was used to sequence whole genomes of two species, *Paederus fuscipes* and *Aleochara (Aleochara) curtula*. Microsatellite loci were selected with flanking primer sequences. Specimens of *P. fuscipes* and *A. curtula* were collected from three localities, respectively. Genetic diversity and population structure were analyzed using the newly developed microsatellite markers.

**Results** The number of alleles ranged 5.727–6.636 (average 6.242) and 2.182–5.364 (average 4.091), expected heterozygosity ranged 0.560–0.582 (average 0.570) and 0.368–0.564 (average 0.498), observed heterozygosity ranged 0.418–0.644 (average 0.537) in *P. fuscipes* and *A. curtula*, respectively. Population structure indicates that individuals of *A. curtula* are clustered to groups where they were collected, but those of *P. fuscipes* are not.

**Conclusion** Population structures of *P. fuscipes* were shallow. In *A. curtula*, however, it was apparent that the genetic compositions of the populations are different significantly depending on collection localities.

**Keywords** *Aleochara (Aleochara) curtula* · *Paederus fuscipes* · Microsatellite · Genetic diversity · Population structure · Korea

Introduction

Staphylinidae Latreille, the rove beetles, is one of the largest family represented by approximately 63,600 species (Irmler et al. 2018). Among 32 subfamilies of rove beetles, the subfamily Paederinae Fleming contains 5962 species within 225 genera (Anlaş and Çevik 2008). The genus *Paederus* Fabricius, a widespread speciose group, comprises approximately 530 described species, and 128 species in eight subgenera are recorded in Palaeartic region (Assing 2020). The subfamily Aleocharinae Fleming is the largest staphylinid subfamily including approximately 16,200 species (Leschen and Newton 2015). The genus *Aleochara* Gravenhorst is the one of the most speciose genera comprising about 540 species within 19 subgenera (Yamamoto and Maruyama 2016).
Recently, there have been nationwide or local outbreaks of *Paederus fuscipes* (available in: https://imnews.imbc.com/replay/2019/nwdesk/article/5522390_28802.html) and *Aleochara (Aleochara) curtula* (available in: https://www.kyongbuk.co.kr/news/articleView.html?idxno=2010335) in South Korea. These species are known as potential biocontrol agents because they feed on agricultural/hygienic pests. The adults of *Paederus* species are predators feeding on insect pests including moths, aphids and plant hoppers (Khan et al. 2018; Nasir et al. 2012). Paederin, toxic compound synthesized by bacterial symbiont, is secreted from abdomens of *P. fuscipes* (Piel et al. 2004). Dermatitis symptoms by *P. fuscipes* and allied species have occurred worldwide (Gurcharan and Syed 2007; Kerion 1993; Todd et al. 1996). *Aleochara* females exclusively oviposit nearby pupated fly maggots, whose larvae trace and infiltrate into pupae (Maus et al. 1998). Although attacks to tourists caused by *A. curtula* have been reported in South Korea, damage in other countries is unknown.

The “Paederus dermatitis” damages have been reported continuously (Kim et al. 1989; Lim et al. 1996) and abundance of *A. curtula* also increased in 2018–2019. Despite their continuous outbreaks, studies of genetic diversity or genetic structure remain extremely limited. Microsatellites are DNA sequences consisting of 1–6 tandemly repeated nucleotides and can be used as informative DNA markers (Kwak et al. 2020). Several research about the genetic diversity of populations using microsatellite markers have been conducted for the use of conservation for endangered species (Kim et al. 2019; Kwak et al. 2020) or monitoring the domesticated organisms including crops and livestock (Biswas et al. 2020; Zhang et al. 2021).

In this study, we developed microsatellite markers of *P. fuscipes* and *A. curtula* for the first time. Genetic diversity was analyzed by genotyping polymorphic alleles using the newly developed microsatellite markers. We also visualized population structure. This work can provide insights for identifying characteristics of certain population within the species.

**Material and methods**

**Collecting samples and DNA extraction**

The specimens of *Paederus fuscipes* were collected manually on wet flatland including rice paddies from Taean-gun, Chungcheongnam-do [TA, 2.VII.2020], Suncheon-si, Jeollanam-do [SC, 21.V.2020], and Yesan-gun, Chungcheongnam-do [YS, 16.IV.2020]. Baited pitfall traps were used for sampling specimens of *Aleochara (Aleochara) curtula* from Muju-gun, Jeollabuk-do [MJ, 4–11.VI.2020], Gyeongju-si, Gyeongsangbuk-do [GJ, 20–27.VII.2018], and Jeongeup-si, Jeollabuk-do [JE, 1–8.IV.2020] (Table S1, Fig. 2). Genomic DNA samples were extracted from the entire body using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany).

**DNA sequencing and Microsatellite identification**

After extracting the genomic DNA of *P. fuscipes* and *A. curtula*, we checked the quality of DNA by 1% agarose gel electrophoresis and PicoGreen® dsDNA Assay (Invitrogen). DNA library was prepared according to Illumina TrueSeq DNA PCR-Free Library prep protocol. The quality of the libraries was verified by capillary electrophoresis (Bioanalyzer, Agilent). The library was clustered on the Illumina cBOT station and sequenced paired end for 101 cycles on the Novaseq 6000 sequencer according to the Illumina cluster and sequencing protocols.

Appropriate microsatellite loci from the genome sequence were searched using the QDD program (Meglécz et al. 2010), and flanking primer sets were also detected. Appropriate primer sequences were selected with high effective diversity according to the microsatellite development workflow (Lepais et al. 2020).

**Development of microsatellite markers**

The forward primers used to amplify the microsatellite loci was labeled with a fluorescent dye, 6FAM. To validate the selected primers, Polymerase chain reaction (PCR) was performed in a total volume of 20 μl containing 0.4 μl genomic DNA, 8 μl each for forward and reverse primers and 10 μl BioFACT™ Lamp Taq PCR Master Mix 2 (BIOFACT, Daejeon, Korea), with an initial activation of 5 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at the locus-specific annealing temperature, 30 s at 72 °C and a final extension at 72 °C for 5 min using a Thermal Cycler (VeritiPro, Marsiling, Singapore). Genotyping the obtained alleles was performed using ABI PRISM 3730XL Analyzer and GeneMapper® Software Version 4.0.

**Data analysis**

Genetic diversity parameters including the average number of alleles (Ad), expected heterozygosity (He), observed heterozygosity (Ho), and polymorphic information content (PIC) were analyzed using PowerMarker V3.25 (Liu and Muse 2005), which was also conducted for reconstructing UPGMA and NJ trees. GenAlEx 6.503 (Peakall and Smouse 2012) was used for AMOVA (analysis of molecular variance), PCoA (principal coordinates analysis), and calculating genetic distances (Fst) among populations.

The population structure was analyzed using STRUCTURE RE 2.3.4 (Porras-Hurtado et al. 2013). To predict the optimum K value, the appropriate number of clusters constituting
the structure, the program was run in three repetitions for each K from 2 to 10, with a burn-in period of 100,000 and 200,000 Markov chain Monte Carlo (MCMC) iterations. CONVERT (Glaubitz 2004) was used to transfer text files to input data for STRUCTURE 2.3.4. software. Appropriate K values were determined using the web-based software STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Results

Development of the microsatellite markers

NGS produced 109,081,018 reads and 16,471,233,718 bp as total sequence from Paederus fuscipes. A total of 22,185,404,861 bp (146,923,211 reads) were obtained from Aleochara (Aleochara) curtula. The numbers of assembled contigs in P. fuscipes and A. curtula were 1,683,526 (totally 1,335,234,806 bp) and 63,105 (totally 128,993,284 bp), respectively. Eleven primers which amplified the gDNA of specimens were determined to be markers. The PIC (Polymer Information Content) value ranged 0.033–0.841 and 0.122–0.989 (Table 1). UPGMA also shows scattered plots for P. fuscipes but clustered plots for A. curtula (Fig. 1c, f). The results of AMOVA suggest relatively high genetic differentiation with 26% of variation among populations of A. curtula, while P. fuscipes shows low population differentiation (Table 2). The highest genetic distance ($F_{ST}$) was 0.005 between TA and SC in P. fuscipes, 0.348 between MJ and GJ in A. curtula (Fig. 2).

Discussion

We amplified 11 microsatellite loci from Paederus fuscipes and Aleochara (Aleochara) curtula, respectively. Among the newly developed markers, PF-005, PF-006, AC-002 are weakly informative (PIC < 0.25), PF-001 is quite informative (0.25 < PIC < 0.5), and the others are highly informative (PIC > 0.5) (Tables S2, S3) according to Botstein et al. (1980).

The $H_e$ of SC was relatively higher than the other populations, and GJ was the minimum. The minimum $H_e$ was GJ, however, the maximum was JE (Table 1). Considering the $H_e$ is calculated theoretically assuming random mating, it cannot be able to exactly match or corresponding the heterozygosity within actual populations and gene diversity (Gregorius 1978).

Genetic diversity among populations of P. fuscipes was almost identical and it showed shallow structure both K = 7 and K = 4 (Fig. 1b). Although the physical distances among three localities, genetic distances were extremely low (Fig. 2). It suggests that gene flows of P. fuscipes individuals were less limited and there were robust genetic communications. Populations of A. curtula showed strong structure. Estimated K values supported two genetic groups (Fig. 1d, e) which coincide with the pie chart of Fig. 2 (right). Especially GJ (Gyeongju), the region where the outbreak occurred, indicated low genetic diversity and extremely high genetic distances against the others.

| Table 1 Genetic diversity of Paederus fuscipes and Aleochara (Aleochara) curtula populations |
|-----------------|-----|-----|-----|-----|-----|
| Species         | ID  | N   | Ad  | $H_e$ | $H_o$ |
| P. fuscipes     | TA  | 30  | 5.727 | 0.560 | 0.497 |
|                 | SC  | 30  | 6.364 | 0.582 | 0.458 |
|                 | YS  | 29  | 6.636 | 0.569 | 0.460 |
|                 | Average | 6.242 | 0.570 | 0.472 |
| A. curtula      | MJ  | 30  | 5.364 | 0.564 | 0.551 |
|                 | JE  | 30  | 4.727 | 0.563 | 0.644 |
|                 | GJ  | 30  | 2.182 | 0.368 | 0.418 |
|                 | Average | 4.091 | 0.498 | 0.537 |

$N$ number of samples, $Ad$ mean number of alleles, $H_e$ expected heterozygosity, $H_o$ observed heterozygosity
Conclusion

Geographically isolated, individuals of GJ are restricted to communicate with those of other localities. In addition, the evolution within genus *Aleochara* took place rapidly. Molecular phylogeny suggested that the diverse species originated in relatively short period (Maus et al. 2001). Our results provided basic data for a quick monitoring technique dealing with future outbreaks, but also discovering potential subspecies of *A. curtula*. It is expected that microsatellite markers can be applied to evaluate genetic diversity of more than population level within the family Staphylinidae.

Fig. 1 Population structures. a, d Appropriate K values calculated by STRUCTURE HARVESTER. b, e Estimated population structures by STRUCTURE. c, f PCoA results. a–c *Paederus fuscipes*. d–f *Aleochara* (*Aleochara*) *curtula*
Table 2  AMOVA result of two species

| Species     | Source       | df | SS     | MS     | Est. Var | %   |
|-------------|--------------|----|--------|--------|----------|-----|
| *P. fuscipes* | Among Pops   | 2  | 8.230  | 4.115  | 0.005    | 0%  |
|             | Among Indiv  | 86 | 326.284| 3.794  | 0.593    | 18% |
|             | Within Indiv | 89 | 232.156| 2.608  | 2.608    | 81% |
|             | Total        | 177| 566.671| 3.207  |          | 100%|
| *A. curtula* | Among Pops   | 2  | 118.411| 59.206 | 0.946    | 26% |
|             | Among Indiv  | 87 | 210.4  | 2.418  | 0        | 0%  |
|             | Within Indiv | 90 | 241    | 2.678  | 2.678    | 74% |
|             | Total        | 179| 569.811| 3.624  |          | 100%|

*df* degree of freedom, *SS* sum of squares, *MS* mean squares

Fig. 2  The average cluster assignments at each location and genetic distances among populations. Habitus of examined species are provided. Left: *Paederus fuscipes*. Right: *Aleochara (Aleochara) curtula.*

Genetic structures are displayed as pie charts. \( F_{st} \) values between TA–YS and SC–YS was calculated to be 0 because of a few individual samples.

**Supplementary Information**  The online version contains supplementary material available at https://doi.org/10.1007/s13258-022-01293-2.

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**Declarations**

**Conflict of interest**  Yeon-Jae Choi, Jeesoo Yi, Chan-Jun Lee, Ji-Wook Kim, Mi-Jeong Jeon, Jong-Seok Park, and Sung-Jin Cho declare that they have no conflict of interest.

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References

Anlaş S, Çevik IE (2008) Faunistic studies on Paederinae (Coleoptera: Staphylinidae) in Manisa province, Turkey. Mun Ent Zool 3:665–674
Assing V (2020) On the taxonomy and zoogeography of Paederus V. Two new species from Laos and China, a new synonymy, new subgeneric assignments, and new records from the Palaeartic region (Coleoptera: Staphylinidae: Paederinae). Acta Mus Morav Sci Biol 105:91–102
Biswas MK, Bagchi M, Biswas D, Harikrishna JA, Liu Y, Li C, Sheng O, Mayer C, Yi G, Deng G (2020) Genome-wide novel genic microsatellite marker resource development and validation for genetic diversity and population structure analysis of banana. Genes 11:1479
Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331
Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361
Glaubitz JC (2004) CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol Ecol Notes 4:309–310
Gregarious H-R (1978) The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math Biosci 41:253–271
Gurcharan S, Syed YA (2007) Paederus dermatitis. Indian J Dermatol Venereol Leprol 73:13–15
Irmler U, Klimaszewski J, Betz O (2018) Introduction to the biology of rove beetles. In: Betz O, Irmler U, Klimaszewski J (eds) Biology of Rove Beetles (Staphylinidae): life history, evolution, ecology and distribution, 1st edn. Springer International Publishing, Cham, pp 1–4
Kerion K (1993) Paederus dermatitis amongst Medical Students in US M, Kelantan. Med J Malaysia 48:403
Khan MM, Nawaz M, Hua H, Cai W, Zhao J (2018) Lethal and sublethal effects of emamectin benzoate on the rove beetle, Paederus fuscipes, a non-target predator of rice brown plant hopper, Nilaparvata lugens. Ecotoxicol Environ Saf 165:19–24
Kim Y-P, Chun I-K, Hur S-G, Ha B-S (1989) Clinical and entomological studies of Paederus Dermatitis. Korean J Dermatol 27:402–411
Kim B, Nakamura K, Tamura S, Lee BY, Kwak M (2019) Genetic diversity and population structure of Lychnis wilfordii ( Caryophyllaceae) with newly developed 17 microsatellite markers. Genes Genomics 41:381–387
Kwak YH, Kim KR, Kim MS, Bang IC (2020) Genetic diversity and population structure of the endangered fish Pseudobagrus brevicorpus (Bagridae) using a newly developed 12-microsatellite marker. Genes Genomics 42:1291–1298
Lepais O, Chancerel E, Bouri C, Salin F, Manicki A, Taillebois L, Dutech C, Aissi A, Bacles CF, Daverat F (2020) Fast sequence-based microsatellite genotyping development workflow. PeerJ 8:1–28
Leschen RA, Newton AF (2015) Checklist and type designations of New Zealand Aleocharinae (Coleoptera: Staphylinidae). Zootaxa 4028:301–353
Lim H-S, Jung C, Kim D-H, Pyun S-H (1996) A study on the epidemic of paederus dermatitis occurred among apartment residents. J Agric Med Community Health 21:13–20
Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129
Maus C, Mittmann B, Peschke K (1998) Host records of parasitoid Aleochara Gravenhorst species (Coleoptera, Staphylinidae) attacking puparia of cyclorrhapheous Diptera. Dtsch Entomol Z 45:231–254
Maus C, Peschke K, Dobler S (2001) Phylogeny of the genus Aleochara inferred from mitochondrial cytochrome oxidase sequences (Coleoptera: Staphylinidae). Mol Phylogenetics Evol 18:202–216
Meglécz E, Couston C, Dubut V, Gilles A, Malausa T, Pech N, Martin J-F (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403–404
Nasir S, Akram W, Ahmed F (2012) The population dynamics, ecological and seasonal activity of Paederus fuscipes Curtis (Staphylinidae; Coleoptera) in the Punjab, Pakistan. AFGBEE Proc 4:36–41
Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539
Piel J Jr, Høfer I, Hui D (2004) Evidence for a symbiosis island involved in horizontal acquisition of pederin biosynthetic capabilities by the bacterial symbiont of Paederus fuscipes beetles. J Bacteriol 186:1280–1286
Porras-Hurtado L, Ruiz Y, Santos C, Phillips C, Carracedo A, Lareu MV (2013) An overview of STRUCTURE: applications, parameter settings, and supporting software. Front Genet 4:98
Todd RE, Guthridge SL, Montgomery BL (1996) Evacuation of an Aboriginal community in response to an outbreak of blistering dermatitis induced by a beetle (Paederus australis). Med J Aust 164:238–240
Yamamoto S, Maruyama M (2016) Revision of the subgenus Aleochara Gravenhorst of the parasitoid rove beetle genus Aleochara Gravenhorst of Japan (Coleoptera: Staphylinidae: Aleocharinae). Zootaxa 4101:1–68
Zhang X, He Y, Zhang W, Wang Y, Liu X, Cui A, Gong Y, Lu J, Liu X (2021) Development of microsatellite marker system to determine the genetic diversity of experimental chicken, duck, goose, and pigeon populations. Biomed Res Int 2021:1–14

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