Floral structures affect on pollination events of sandalwood in four landraces along landscape gradients in Gunung Sewu, Java, Indonesia

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Abstract. Sandalwood, one of the most economic-endangered species, was the origin to the south-eastern islands of Indonesia, but is recently existed as new landraces in several community forests in Gunung Sewu Geopark, Indonesia. All of landraces consisted of three floral variants (YBF, refers to “yellow big flower”; RBF, “red big flower”; and RSF, “red small flower”, respectively). Our previous findings reported differences on floral structures among variants. In this study we further analyzed these variants differences and its consequence on pollination events, among four landraces represented each geographical zone in Gunung Sewu Geopark, in the 2016 flowering periods. The flowering traits of each variant and landrace were measured. Floral visitors diversity, visitation rate and pollination behaviour were observed in each of population and floral variants. Results showed that flower abundance, visitor diversity and visitor abundance varied with sites but were similar among floral variants. Flowers abundance was strongly correlated to the pollinator visitation rate. Visitors diversity varied with sites due to the differences on flower abundance, environmental condition and altitude. The cooler and wetter site was visited more by Dipterans and Hymenopterans. The warmer, drier and lower sites were visited more by Lepidopterans and Dipterans. Sites with fewer flowers received less visitor diversity and abundance. Visitors preference to a given floral variant might be attributed to the differences on floral structures, sizes and colors. Lepidopterans belong to the moth groups (Arctiidae, Hesperiidae and Noctuidae) preferred to visit the yellow flowers of YBF variant, while those belong to the butterflies groups (Papillionidae, Nymphalidae and Pieridae) preferred the red flowers of RBF and RSF. All six families of Hymenopterans preferred to visit the red flowers with bigger size, RBF. In contrast, all four families of Coleopterans, as well as the four families of Hemipterans, preferred the yellow flowers of YBF. All four families of Dipterans made similar visits to both RBF and YBF which have bigger flowers, while the visitation was less in RSF.

1. Introduction
The Outer Arc of Banda Islands, now is the south-eastern parts of Indonesian archipelagos, is proven as the centre of origin of sandalwood worldwide [1-4]. An economic-important species Santalum album Linn (Santalaceae), herein after referred to sandalwood, bears heartwood containing 1.5 to 5% of β-santalol, a strong, specific fragrances of secondary metabolites. The sandals heartwood has been widely used for carving, art, religious and medicinal purposes. Sandalwood oil is the prime sources for
cosmetics, perfumes and aroma-therapy, and was considered containing anti-melanoma compounds [3-4]. Considering the significant habitat degradation and loss, this species was first listed as vulnerable in the IUCN Red List of Endangered Species in 1994 [5]. Since the habitat loss has significantly increased (more than 100% increment) within less than a decade or three generations, the vulnerable status might be raised up to endangered, critically endangered or even extinct in the wild [6].

Despite a rapid degradation in their origin in the South-eastern islands of Indonesia, new landraces of sandalwood found in Gunung Sewu Geopark, a 1300 km² mountainous limestone zones extended along the Java island [7]. These new landraces may provide materials for any of reintroduction and rehabilitation efforts. Sandalwood in Gunung Sewu extended along geographical gradients under various types of landscapes. Our preliminary studies reported that each landrace in Gunung Sewu consisted of at least three sandal variants (YBF, refers to “yellow big flower”; RBF, “red big flower”; and RSF, “red small flower”, respectively) differed by floral structures and sexual organs longevity [8].

Floral traits, which cover several aspects of floral structures [9;10; 11 da Cruz et al 2006], the structure and arrangement of sexual organs [11, 12, 13], and plant size and age [14] determine floral display in population. The differences on floral display may resulted in the differences of pollinators and pollinator behaviour [10, 15]. In sandalwood, it may also resulted in different mating systems, pollination success and reproductive outputs [16, 17, 18 26]. A mating incompatibility due to floral sexual organ differences has reported in S. album, S. lanceolatum and S. spicatum in Western Australia [13], S. album in India ([26 ; 17,18], and S. lanceolatum in Australia [20].

Our observation on the effect of sandal's floral variant differences to the pollination events was first conducted in Bleberan, one of sandalwood landrace in the Middle Zone of Gunung Sewu [21]. Our previous findings in Bleberan landrace showed that visitor preference to a given variant might be attributed to the differences of floral color and size. In this recent study, we compare floral variants and its effects on pollination events, among four landraces along environmental gradients in Gunung Sewu Geopark, in the 2016 flowering periods. Flowers were counted, and the visitors diversity, visitation rate and pollination behavior were observed in each of landrace and floral variant.

2. Materials and Method

2.1. Study sites
This study compared four landraces along geographical and environmental gradients in Gunung Sewu, which were representing distinctively different population structures: one population, the basin of Bleberan in the Middle Zone, two (the highland of Nglanggeran and the lowland of Bejiharjo) in the Northern Zone, and one (the karst area Petir) in the Southern Zone, respectively. Each of population is separated by 25 to 40 km. These sites are at different altitude, experienced different climatic regimes and having clear ecological differences [8,22].

Nglanggeran mountain (710-750 m asl) is a part of Nglanggeran Mountainery Formation in the Northern Zone of Gunung Sewu. Now existed as the mountainary landscapes, strong undulating, characterizing tropical mountain ecosystems under Am climatic type according to Koeppen’s. In the dry season 2016, this site received abundant rainfall (2280 mm yearly rainfall on more than 6 rainy months) and experienced lowest temperature (31.640C), highest relative humidity (70.39%) and highest soil moisture (28.74%), respectively. Soils are dominated by latosols with volcanic and sediment rocks, some with deeper solum. Sandalwood was first documented in 1970’s. Recently occurred in groups of stands across the Nglanggeran mountain regions in 79.3 ha of width, in association with the tropical mountain vegetation. Habitat dominated by the association of naturally regenerated mahogany, Gliricidea sp, and several Garcinia and Eugenia families.

Bejiharjo (150-180 m asl) is a part of Sambipitu Lowland Formation in the Northern Zone of Gunung Sewu. Now existed as the open dry-rocky hilly landscapes with caves and ground-rivers below, representing the dryland-arid ecosystems under strong Aw climatic type according to Koeppen’s. In the dry season 2016, this most dry population received lowest rainfall (1511 mm yearly rainfall on less than 6 rainy months), highest temperature (40.51°C), lowest relative humidity (31.94%) and lowest soil moisture (21.32%), respectively. Soils are the association of red mediterrans and black grumosols with limestone rocks, mostly with the shallow solum. Sandalwood is a remnant of the 1970’s planted stands,
which undergo on-going heavy exploitation, urban and cave-tourism activities since 1990’s. Now existed as a small-fragmented group of stands in only 9.6 ha of width, occurred in an open dry-rocky hills above the caves and ground-rivers. Sandalwood grew in an association with cajuputi and acacia regenerated from commercial plantation nearby. Younger sandal trees were largely derived from root suckers. Sites dominated by dryland herbs such as grasses and Eupatorium sp.

Bleberan (150-170 m asl) is a part of Wonosari Basin Formation in the Middle Zone of Gunung Sewu. Now existed as the catchment area of the ancient subterranean Oya River at the lowland basin landscapes. Representing the tropical lowland ecosystems, in the intermediate between Aw and Am type. This population located in a basin area that was receiving abundant of rainfall (2346 mm yearly rainfall on 2 to 6 rainy months). Therefore, even it possessed the intermediate of Am and Aw climatic type with high temperature (34.37°C) and light intensity, it was able to maintain relatively higher soil moisture (27.33%) and relative humidity (58.36%). Soils are the association of red mediterrans and black grumosols with limestone rocks, mostly with the deeper solum. Sandalwood was first documented in 1970’s along the catchment area of the ancient subterranean Oya River, at the lowland basin of middle zone. Recently, sandalwood comprised more than 52 ha, along the riparian catchment area and nearby, in association with the tropical lowland forest vegetation which is consisted of more diverse vegetation including teak, mahogany, Gliricidiea sp, Schleichera sp, cajuputi and acacia. Population is surrounded by several ex situ conservation areas which are sharing the same river.

Petir (70-100 m asl) is a part of Wonosari-Punung Karst Formation in the Southern Zone of Gunung Sewu. Now existed as the karst hilly landscapes with open dry-rocky hills, strong undulating, characterizing the dry rocky-limestone ecosystems under strong arid Aw climatic type according to Koeppen’s. In the dry season 2016, this site received lower rainfall (1653 mm yearly rainfall on less than 6 rainy months), much higher temperature (39.540°C), lower relative humidity (37.54%) and lower soil moisture (23.88%), respectively soils are latosols with limestone rocks. Solum is deeper at the basins, but very shallow at the limestone-rocky hills. Sandalwood was first documented in 1960’s in karst hilly areas, recently covering more than twenty open dry-rocky hills in more than 78 ha of width. Mature plants were mostly derived from root suckers; highly clonalized. In the open-undulating areas, sandalwood grew in association with dry rocky-limestone vegetation including acacia and cajuputi, but more dominated by shrubs and herbs such as grasses and Eupatorium sp.

2.2. Study species
We observed sandalwood in Gunung Sewu which were divided into three variants (YBF, refers to “yellow big flower”; RBF, “red big flower”; and RSF, “red small flower”, respectively) differed by the floral traits (floral color, structures, arrangements and the longevity of sexual organs). Our previous findings reported that the RSF and RBF are dominated by maroon and reddish color, while YBF is more yellowish to orange. The RBF and YBF have bigger perigonium, longer size of sexual organs with similar/lower position of stylus to the stamens, and possess shorter period of longevity. RSF flowers are smaller with stylus are similar/higher than the anthers, and possess longer longevity [8]. Flowering varied among sites due to the environmental factors such as altitude, soils and climatic differences. Sandalwood in lower altitude, drier and warmer sites flowered earlier and shorter [23]. However, the floral and flowering differences among variants were considered to be under genetic controls. YBF flowered earliest, RBF was the latest, and RSF has longest flowering period (more detail data on floral structures, sexual organs longevity and flowering phenology of these three variants are available at [8, 21, 23]).

2.3. Pollinators and pollination observation
Observation was conducted in approximately 60 hours at each of landrace, during the peak flowering period in the dry season of 2016’s. Pollinators activity was quantified in each of landrace, where 18 observers simultaneously monitored pollination activities at nine points, with three flowering trees per point (total N = 3 points x 3 variants x 3 flowering trees = 27 trees per landrace), for periods of 12 hours per day (started from 05:00 am to 05:00 pm)[24,25]. over a total of five days of peak flowering period [8,21, 23]. The observation interval at each of landrace varied from 48 to 60 hours per flowering period, however was fewer than 60 hours in the highland site on account of heavy rain at this higher elevation.
The total number of single flowers, which refers to the sum of all flowers produced by the nine sampled trees of each variant at each of site, was counted. The type and number of flower visitors were recorded. Visitation rate, which refers to the number of visits per given time period [11] was estimated for each type of flower visitors. The floral visitors were captured and dry mounted for insect identification and pollen load observation in the Entomological Laboratory, Faculty of Agriculture, Universitas Gadjah Mada.

3. Result and Discussions

3.1. Pollinators and polination activity

3.1.1. Pollinators diversity and visitation rate.
In over 240 hours of field observations made during flowering period in the dry season of 2016, the number of visits totalled 1735 for a sum total of 19,113 single flowers (four landraces, 12 flowering trees each). Site with fewer flowers received less visitor diversity and abundance. Flower abundance, visitor diversity and visitor abundance varied with sites but were similar among floral variants (Table 1; Figure 1).

Visitors diversity varied with sites due to the differences on flower abundance, environmental condition and altitude. The cooler and wetter site was visited more by Dipterans and Hymenopterans. The warmer, drier and lower sites were visited more by Lepidopterans and Dipterans.

Visitor preference also varied with floral variants, which might be attributed to the differences on floral color and size. Visitation in the yellow flowers of YBF variant was dominated by coleopterans and hemipterans, while the red flowers of both RBF and RSF were visited more by hymenopterans. The dipterans and lepidopterans visited both yellow and red flowers in a similar visitation rate. The bigger flowers of RBF and YBF received more visits than RSF, the smaller ones (Figure 1a).

Family diversity and abundance of visitors also differed by sites. The Coleoptera1 and Coleoptera2 (Coleopterans), Muscidae (Dipterans), Scoliidae and Megachilidae (Hymenopterans), and Phyrrocidae, Scutelleridae and Cicadellidae (Hemipterans), were observed only in the highland site and not in the lowland ones. In contrast, the Papillionidae (Lepidopterans), Diptera1 and Calliphoridae (Dipterans), and Platasipidae (Hemipterans) were found only at the lowland sites (Figure 1b to 1f).

Visitors preference also determined by the floral structures and colors. Lepidopterans belong to the moth groups (Arctiidae, Hesperiidae and Noctuidae) preferred to visit the yellow flowers of YBF variant, while those belong to the butterflies groups (Papillionidae, Nymphalidae and Pieridae) preferred the red flowers of RBF and RSF (Figure 1a). All six families of Hymenopterans (Figure 1d) preferred to visit the red flowers with bigger size, RBF. In contrast, all four families of Coleopterans (Figure 1e), as well as the four families of Hemipterans (Figure 1f), preferred the yellow flowers of YBF. All four families of Dipterans made similar visits to both RBF and YBF which have bigger flowers, while the visitation was less in RSF (Figure 1b) (some of the data of Nglanggeran landrace has also been published in Ref [21].
Table 1. Flowers abundance and pollination rate in the three variants of sandalwood among four landraces in Gunung Sewu. F ratios and P values were resulted from test of analysis of variance (ANOVA, LSD test) performed among parameters within populations and within floral variants. Asterisked values indicated significant differences at P < 0.05.

| Populations and floral variants | Flower abundance (individuals/5 days) | Pollination rate |
|---------------------------------|----------------------------------------|------------------|
| **Among populations**           |                                        |                  |
| Nglanggeran                     |                                        |                  |
| - Red-big flower var.           | 1087                                   | 71               |
| - Yellow-big flower var.        | 2078                                   | 72               |
| - Red-small flower var.         | 1463                                   | 47               |
| Average                         | 1543                                   | 63               |
| Bejiharjo                       |                                        |                  |
| - Red-big flower var.           | 2114                                   | 93               |
| - Yellow-big flower var.        | 2088                                   | 95               |
| - Red-small flower var.         | 1847                                   | 72               |
| Average                         | 2016                                   | 87               |
| Bleberan                        |                                        |                  |
| - Red-big flower var.           | 971                                    | 57               |
| - Yellow-big flower var.        | 1351                                   | 67               |
| - Red-small flower var.         | 1038                                   | 41               |
| Average                         | 1120                                   | 55               |
| Petir                            |                                        |                  |
| - Red-big flower var.           | 951                                    | 94               |
| - Yellow-big flower var.        | 1372                                   | 89               |
| - Red-small flower var.         | 1322                                   | 71               |
| Average                         | 1215                                   | 85               |
| F stat.                         | 0.0004*                                | 0.048*           |
| P value                         | 9.46*                                  | 3.14*            |
| **Among floral variants**       |                                        |                  |
| Red-big flower var.             | 1281                                   | 79               |
| Yellow-big flower var.          | 1722                                   | 80               |
| Red-small flower var.           | 1417                                   | 58               |
| F stat.                         | 2,040904                               | 2,351276         |
| P value                         | 0,154894                               | 0,119826         |
Figure 1. The visitors diversity at the Order (a) level, and at the family level within the Order of Dipterans (b), Lepidopterans (c), Hymenopterans (d), Coleopterans (e) and Hemipterans (f), respectively, in three sandalwood variants in four landraces in Gunung Sewu.

* Some of the families are still unidentified and hereby marked as Diptera1 (Dipterans), Coleoptera1, Coleoptera2, and Coleoptera3 (Coleopterans)

4. Conclusions
Flower abundance, visitor diversity and visitor abundance varied with sites but were similar among floral variants. Flowers abundance was strongly correlated to the pollinator visitation rate. Visitors diversity varied with sites due to the differences on flower abundance, environmental condition and altitude. The cooler and wetter site was visited more by Dipterans and Hymenopterans. The warmer, drier and lower sites were visited more by Lepidopterans and Dipterans. Sites with fewer flowers received less visitor diversity and abundance. Family diversity and abundance of visitors also differed
by sites. The Coleoptera1 and Coleoptera2 (Coleopterans), Muscidae (Dipterans), Scoliidae and Megachilidae (Hymenopterans), and Phyrrocoidea, Scolitidae and Cicadellidae (Hemipterans), were observed only in the highland site and not in the lowland ones. In contrast, the Papilionidae (Lepidopterans), Diptera1 and Calliphoridae (Dipterans), and Platasipidae (Hemipterans) were found only at the lowland sites.

Visitors preference to a given floral variant might be attributed to the differences on floral structures, sizes and colors. Lepidopterans belong to the moth groups (Arctiidae, Hesperidae and Noctuidae) preferred to visit the yellow flowers of YBF variant, while those belong to the butterflies groups (Papilionidae, Nymphalidae and Pieridae) preferred the red flowers of RBF and RSF. All six families of Hymenopterans preferred to visit the red flowers with bigger size, RBF. In contrast, all four families of Coleopterans, as well as all four families of Hemipterans, preferred the yellow flowers of YBF. All four families of Dipterans made similar visits to both RBF and YBF which have bigger flowers, while the visitation was less in RSF.

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