CHAPTER 6

CONCLUSION AND DISCUSSION

Floral Development and Morphology, Flowering Phenology and Anthesis Process, and Nectary Studies

1. Floral Morphology

Afgekia sericea Craib produces long raceme, the most common inflorescence among legumes (Tucker, 1987), bearing a large number of flowers. Like other fabaceous species, has least variation in the floral ground plan, the floral structure of *A. sericea* is a specialized bilaterally symmetrical papilionoid flower. Tucker (1987) and Judd et al., (1999) pointed out that most of the Fabaceae have a recognizable papilionoid corolla, although they show extremely innumerable variation in such features as connation, twisting, elongation, claw formation, beak, pits projections, sculpturing, and coloration. The pea flower is the most striking example of a highly zygomorphic, polypetalous corolla. In *A. sericea*, the greenish yellow odd rear petal, with overlaps all the others, is both the largest and the most conspicuous (Bhattacharyya and Johri, 1998). It is known as the "standard" or the "vexillum", this is because it is often the only petal, which is directed upwards and spreads flat as the flower opens, whilst the other petals remain more or less folded up. The lower two petals are united into a "keel", which enclose the stamens and the gynoecium, and which commonly functions along with the two lateral petals or "wings". The flowers of *A. sericea* are of similar construction. The specialized papilionaceous corolla type has a significant role in various pollination mechanisms. Basically, the conspicuous vexillum functions as a visual attractant or the main optical display organ and is thus frontally exposed and contains a nectar guide of colors, which is dark purple in *A. sericea*, contrasting with the more peripheral region (Zomlefer, 1994). Nectar guides may be even more conspicuous and diverse in the ultraviolet range of the spectrum, with UV-absorbing marks mostly in the floral center; wing petals, deep purple differ from white keel in this species, may also be included (Kay, 1987). However Kay (1987) also found that nectar guides have not been found in bird-pollinated taxa. The keel (Zomlefer, 1994), together with the wings serves as a landing platform for visiting insects, which are predominantly Hymenoptera (Endress, 1994; Weberling, 1992), and the keel encloses and protects the stamens and stigma. The purplish red in color sepals are united into a tubular structure, which is an additional means to provide firmness to the complicated pollination apparatus of petals, stamens and carpel. Thus, the pollinate flower as a whole is an extremely elaborate construction by the synorganization of all its parts (Endress, 1994). In *A. sericea*, the lateral fusions of filaments are occurred. In this species, either the filaments of all ten stamens are united into tube surrounds the gynoecium which composed of one simple pistil, or else only nine of them combine to form a channel which is open above and is covered by the tenth stamen left in isolation and lastly fused to form pseudo-monadelphy according to the stamens are combined into one bundle (Endress, 1994).

2. Floral Development

Since development is programmed, the orientation of flowers is usually predictable. The inflorescence meristem of a simple raceme, the most common type among legumes, of *A. sericea* produces helical phyllotaxy bracts in sequence, each of which subtends a single flower. This result is consistent with many previous studies (Crozier and Thomas, 1993; Hirsch et al., 2002; Kantz and Tucker, 1994; Mansano, Tucker, and Tozzi, 2002; Tang and Skorupska, 1997; Tucker, 1987, 1993. 1994; Tucker and Kantz, 1997; Washburn and Thomas, 2000). Many workers noted that in some Fabaceae and Caesalpiniaceae two bracteoles are formed (Nemoto and Ohashi, 1996; Tucker, 1996, 1998, 2001, 2002a, 2002b, 2002c) although there are not always present in legumes. Flowers are strongly zygomorphic from the times the first organs, the sepals, are initiated, the degree of zygomorphy seen at anthesis is caused in part though initiation of organs, and in part by differential growth late in development (Tucker, 1987). In support of previous research (Tucker, 1987), this study found that the flowers are pentamerous, with member of whorl alternating radially with those of adjacent whorls. Initiation of floral organs can be considered among whorls and within whorls. This finding is similar to results from many previous investigations (Crozier and Thomas, 1993; Hirsch et al., 2002; Kantz and Tucker, 1994; Mansano, Tucker, and Tozzi, 2002; McMahon and Hufford, 2002; Tang and Skorupska, 1997; Tucker, 1987, 1993. 1994, 2002a; Washburn and Thomas, 2000). The vertical order of initiation, among whorls, is acropetal. The order of initiation of organs within whorls, are initiated unidirectionally from the abaxial to adaxial side. Members of a whorl are formed in succession, starting with the one abaxial or two toward the abaxial side of the floral apex, followed by the initiation of lateral organs, and lastly by one adaxial or two toward the adaxial side. In other words, members of each whorl are of different ages, although they quickly become uniform in size later on. In agreement with Tucker (1987), there is slightly overlapped among initiations of different kinds of organs.

Fusion produces many of the important features in this species such as calyx tube, stamen tube, and fused keel petals. There are two types of fusion, post genital fusion of originally free margins and meristem fusion. The first type of marginal fusion or edge-to-edge fusion between originally free organs found in fused keel, the keel petals fuse by appression of the epidermal layers so there is and external ridge, and fusion between the margins of the originally open carpel to form the simple carpel; characteristic of Family Fabaceae (Bell and Hemsley, 2000) and also in this species; generally of the type involving appression, either of the margins or submarginal adaxial surfaces (Tucker, 1987). The nine-stamen tube has fused with the vexillary stamen to form a continuous tube, which is then turned into the pseudo–monadelphous stage also fuses in this way. The meristem fusion involves no originally free margins. This type produces such features as calyx tubes, and in the fusion of first 9 stamens in diadelphous stage.

3. Flowering Phenology and Anthesis Process

3.1 Events in the single inflorescence

In this study it was found that, average full opening cycle of inflorescence takes 68.60 $\pm$ 8.74 days, bract of all florets fell before all anthers dehiscence, which occurred before timing of flower opening. Flowering time of day was related with plant visitors, which will be mentioned more in visitor behavior study. The finding from this study is also similar to the results from the previous investigations by Momose et al. (1998) that pollination systems were related with flowering time of day, reward and floral shape.

3.2 Dynamics of flowering of the whole plant

Flowering magnitude of inflorescences from floral bud until the last floret blooming takes 13 weeks. On the 7^{th} to 10^{th} week all inflorescence are in bloom. The period of time in this study is longer than in the previous study, which was only about 10 weeks; in this study time for young floral bud development was included.

3.3 Flowering course of the whole population

Flowering phenology of plants in a fixed area was monitored. This study highlights the various perspectives from which floral phenology can be viewed. The study population of this study has a long flowering period. At a population level the flowering distribution was not markedly skewed. Larson and Barrett et al. (1996) pointed out that if the floral syndrome of a species is similar to species previously flowering in the community, less skewed distributions might result. In both year experiments, flowering times overlap extensively, but in 2001 was earlier and later than in the year 2002. Flowering phenology varied between years, the results are consistent with Ushimaru and Kikuzawa (1999), that is different phenology may be due to annual precipitation. The long reproductive period, together with the pronounced flowering peak may indicate a satiation strategy for protection against insect attack (Medrano et al., 1999). In support of previous research (Wolfe and Burns, 2001), this study found that a low precipitation rate is a difficult period for this species and as a result more flowers occurs during or immediately following a high precipitation month.

4. Nectary Studies

4.1 Floral nectary structure

Floral nectary in *A. sericea* was present; the flower produces nectar, which is a collar around gynoecium base, or a concave ring between the stamens and the ovary base (Woodland, 2000). As in previous study (Belmonte et al., 1994; Bernardello et al., 2000) the gland is multicellular glandular structures (Dickison, 2000), composed of small isodiametrical cells with thin walls, relatively large nuclei, intensely stained, with dense granular cytoplasm and small vacuoles. In contrast to earlier studies (Belmonte et al., 1994; Bernardello et al., 2000; Rudall, 1992) the glands do not have special vascular supplies, so that the receptacle bundles must fulfill this function. The epidermis does not bear modified stomata, the glands do not have stomata, and the nectar has to be exuded through the rugose cuticle (Fahn, 1989), this finding disagrees with previous research (Belmonte et al., 1994; Bernardello et al., 2000; Langenberger and Davis, 2002). The nectary surface is smooth, in contrast with the ovary, which has pubescence, this data is in agreement with Belmonte et al. (1994).

4.2 Nectar amount and nectar concentration

Overall nectar production ranged from 0.93 - 3.53 µl. This finding agrees with a previous study (Riano et al., 1999; Sampson and Cane, 1999). While in bird pollinated plant it was found that nectar amount was usually higher (Belmonte et al., 1994; Yumoto, 2000) and in wind pollinated plant nectar amount was usually lower (Peeters and Totland, 1999; Weller et al., 1998). Nectar sugar concentration was on average about 65%, this result agrees with a study of Riano et al. (1999), Langenberger and Davis (2002). However this value is higher than in plant that has both nocturnal and diurnal visitors (Slauson, 2000), bumble bee-pollinated (Aizen and Basilio, 1998), and bird-pollinated species (Yumoto, 2000).

4.3 Determination of nectar sugars

Nectar is basically a concentrated sugar solution. The sugars vary from 25-75% by weight depending on the kind of pollinator. Sucrose, glucose and fructose, are the main constituents (Ingrouille, 1992). In this study, the only sugar identified for all samples were the usual three, i.e. sucrose, fructose, and glucose. There was some variability in sugar ratios, however all samples showed that there were sucrose dominant. Bernardello et al. (2000) found that, there were some differences when the pollinator type was compared, with a trend of a higher sucrose proportion in the species pollinated by hummingbirds. This therefore confirms by the suggestion of Endress (1994) that bee-flowers are characteristically sucrose-rich, and hummingbird-flowers are often derived from bee-flowers. The result of the present study supports the notion that *A. sericea* should be bee-pollinated species.

Pollen Viability and Germination, Pollen-Stigma Interaction, and Self-Incompatibility

1. Pollen Viability and Germination

1.1 Pollen viability

In the present study, pollen viability are different at different age. The highest percentage viable pollen was on the anthesis day and a steady reduction of pollen viability was observed when the time passed. Vital staining revealed that the pollen remains viable for approximately 2 days. The finding was supported by Boonkerd (1992), in that study two methods were performed, the results is that pollen staining gave different results from time to time. However, in that work the pollen remains viable for only 1 day and low in viability rate in one method and has a high similarity in the other. In consistent with Pereira et al. (1997), which was found that two pollen viability techniques did not show any distinguishable differences in the same plant. In this study, percent viability indicated by TTC was generally greater than the percent germination. In contrast, the MTT viability assay conducted by Boonkerd (1992) and *in vitro* pollen germination seem likely to be related.

1.2 Pollen germination

Pollen *in vitro* germination in media of different sucrose concentration, it is quite clear that pollens of *A. sericea* require sucrose for their tube germination. Since the significantly high percent germination was found in the medium composed of 50% sucrose, a suitable concentration. In contrast, the low percent germination was observed when the medium composed of lower or higher sucrose. In consistent with Boonkerd (1992), which was found that 30% sucrose was a suitable concentration; however, with other supplements and method are slightly difference. Pollen tubes are undoubtedly interesting cells. They are unusual in growing by tip-extension, and are one of the few types of differentiated higher-plant cell that can be grown successfully in single-cell culture (Derksen et al., 1999; Read et al., 1992).

2. Pollen-Stigma Interaction

2.1 Cytochemical localization of esterases on stigma surface

The stigma is the specialized part of the carpel on which pollen grains are trapped in the act of pollination (Raghavan, 2000; Sanzol, 2003). From this investigation it was revealed that, the white and smooth stigma, becomes wet and receptive before the day of anthesis, and lasted until ca. at noon on the anthesis day, from then onwards, receptivity diminished slowly, until the day after anthesis. In the classification of receptive surfaces (Dulberger et al., 1994), stigma of *A. sericea* should be included in the wet papillate group. According to pollen germination study that will be mentioned after and the definitions of plants breeding system (Dafni, 1992; Proctor et al., 1996; Richards, 1997), sexual system in this species is herkogamy. This finding is differing from dichogamous system in some species (Ramsey and Vaughton (2000). Stigma morphology in *A. sericea* receptive almost without changing, in contrast with some bearing stigmatic lobes species, it was found that stigmatic lobs separated when receptive (Schluter and Punja, 2000).

2.2 Pollen *in vitro* germination related to pollen age

Pollen *in vitro* germination at 16 time intervals from the day before anthesis to the day after anthesis was found that, pollen at 9:00 h on the anthesis day gave the highest percentage germination. In contrast, pollen on the day before and the day after anthesis showed the low percent germination. It is also found that pollen germination and stigma receptive occurred simultaneously. As it was mentioned before, this species is herkogamous, and differ from dichogamous system (Ramsey and Vaughton (2000) and delayed selfing (Kalisz et al., 1999) in some species.

2.3 Localizing pollen tubes in the stigma

Pollen-pistil interaction, by investigation of pollen tube growth *in vivo*, the bagged flowers and selfing flowers with ungerminated pollen grains were found. Pollen on stigmas yielded germination, if occurred, with short tube in artificial crossed, in contrast, stigma from flowers open to pollination by flower visitors gave a long pollen tube. The germinated pollen grains with the pollen tubes indicated successful pollination, as in previous study (Ushimaru and Kikuzawa, 1999). Lichtenzveig et al., (2000) investigated mating in the climbing cacti and found that pollen grains had germinated and their tubes had penetrated the stigmatic surface on selfed and outcrossed pistils. However, there were different in the percentages of pollen grains germinating on the stigma after distinct pollination treatments, a few pollen tubes extended through the base of the style on the self pistils, by that time, numerous pollen tubes were present at the base of the style on outcrossed pistils in some species, this finding is similar to *A. sericea*. However, the finding disagrees with many previous studies (Mahy and Jacquemart, 1998; Sage et al., 1999; Selbo and Carmichael, 1999) in these studies there were no significant differences in pollen tube behavior following cross- and self-pollinations. In contrast, it was found that pollen germination sometime occurred in both self- and crossed-pollination but in difference rate, (Giblin and Hamilton, 1999). It can be concluded that *A. sericea* is a self-incompatible species, on account of no selfed pollens germination on the stigmas, and due to pollen germination feature, natural crossed-pollination should be differed from artificial method.

3. Self-Incompatibility

Self-incompatibility, the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination (McCubbin and Dickinson, 1997; Nettancourt, 1999; Owens et al., 1992). It is a principle genetic system device promoting outcrossing, and it is a specific angiosperm invention, which is lacking in gymnosperms (Weber, 1999). Self-incompatibility is estimated to be present in more than half of all species of angiosperm (Newbigin et al., 1993). Basically, self - incompatibility prevents success self-pollination. It may result from a variety of mechanisms. Self-pollen either may not adhere to the stigma, adhere but not germinate, or germinate but be unable to penetrate or grow down the style (Kuckuck et al., 1991; Richards, 1997). During the course of the presents study, three techniques were used in order to confirm the present of a self-incompatibility system and to obtain information about the type of self-incompatibility.

3.1 Bagging experiments

The bagging experiments proved to be of important because of the deposition of the pollen due to pollinators. The pollen can be deposited on the sigma during flowering stage and the stigma also receptive during the period of time, which theoretically could result in self-fertilization (autogamy). At that time, pollinators usually have visited flower, so a quantity of none-self pollen always deposited on the stigma head. The results of the bagging experiments indicate that *A. sericea* has a very effective self-incompatibility system, which prevent self-fertilization. The chance of geitonogamy can be ruled out completely since flowers from the other inflorescence were investigated in other experiments. This showed the possibility of fertilization between flowers of different inflorescence on the same plant (geitonogamy), which not been occurred in the present study. This finding agrees with a previous study of Weber (1999).

Both the floral traits and the pollination syndrome point to xenogamy, the pollination and fertilization of flowers on different plants: (1) The papilionaceous flower with nectar guide, (2) the onset of anthesis at early morning relate pollinator foraging behavior, (3) the copious production of nectar at the base of anther tube, and the presence and high abundance of diurnal pollinators.

3.2 Aniline blue fluorescence microscopy

The result of this technique confirms the ones obtain from the bagging experiments. The analysis of several hand cross-pollinated flowers showed high percentage of the stigmas with germinated pollen, however, with short pollen tube. The percentage was also high in opened pollination, which flowers showed high percentage of pistil penetrated by the pollen tubes into the style. The inefficiency of both bagged-pollination treatments, and no pollen tube occurred in artificial self-pollination, indicated that this species is a completely self-incompatible. This finding agrees with a previous study of Weber (1999).

3.3 Pollen grains and ovule (P:O) ratio

The general assumption behind Dafni (1992) is that self-fertilizing plants need not produces much pollen. It indicates that pollen-ovule ratios tend to reflect plant breeding systems. The very high P/O ratio of approximately 16,000 pollen grains per ovule suggests xenogamy as the breeding system in *A. sericea.* The result of the present study is similar to many results from previous investigations (Golubov et al., 1999; Larson and Barrett, 1999; Porras and Alvarez, 1999). Gallardo et al. (1994) found the absence of correlation between P/O ratios and the breeding system, a high autofertility was record in a low P/O ratios species, in the other hand in high P/O ratios autofertility is low.

3.4 Conclusion drawn from three techniques

It can be concluded that in *A. sericea.* a gametophytic self-incompatibility system is present: the pollen grains are not germinate in self pollination. Other feather, which is correlated with gametophytic self-incompatibility, is wet stigma (Weber, 1999) also present in this species. According to Richards (1997), the genetic basis of gametophytic self-incompatibility is as follows: Both alleles at the S locus are expressed by the female stigma and style, and one allele is expressed by the pollen grain. Large numbers of different S alleles may coexist in a population and are termed S_1, S_2, S_3, S_4, and etc. If the S allele in the pollen matches one of the S alleles in the female tissue, fertilization will not occur. Consequently, seeds are invariably heterozygous. Two parents can be fully compatible if neither haploid pollen grain shares the female alleles, semi-compatible if only half the pollen grains can effect fertilization or incompatible if each pollen grain shares one of the female alleles (Richards, 1997).

Behavioral Features of The Visitor and Pollinator-Plant Interaction

The structure and size of the flowers point clearly to pollination by specific pollinators. This assumption is confirmed both by the high frequency and the abundance of diurnal active specific visitors that was mentioned before.

Table 6.1 Characters constituting the pollination syndrome of A. sericea.

Characters constituting the pollination syndrome of *A. sericea.*
1. Early morning on set of anthesis.
2. The structure of the flower: a pseudomonadelphous papilionaceous flower.
3. The presentation of nectar guide in the flower and nectar.
4. Nectar presentation on the daytime in sufficient amount.
5. Nectar sugar concentration and composition related to pollinator species.
6. The nectar is concealed at the bases of the staminal tube.

This character is in full agreement with preponderant visits by diurnal pollinators. It was found that A. sericea always visit by only diurnal visitors started as early as the time simultaneously with high receptivity and also high pollen germination rate, coinciding with the onset of early morning, and continued as long as long as all day time. There was a gradual increase in flower visitors over the next hours, reaching a maximum at about 10:00 h and again 15:00 h. after that visitation become low and occurred only sporadically and infrequently. It is of major interest that, in rainy day visitation usually started later and ended earlier. The reason for this might be different in temperature and/or relative humidity, which consequently stimulate or inhibit the activity pattern of visitors (Weber, 1999). The daylong observations revealed that many pollinators occur in sufficiently high numbers and frequency to be considered as legitimate pollinators. The early morning onset of anthesis also includes bird as pollinators. However the flower of A. sericea visited by birds was rare, only one species was observed, furthermore, due to floral morphology, bird cannot pollinate the flower.

The structure of the flower, a pseudomonadelphous papilionaceous flower, the well exposed position. In this species nectar is concealed, in addition pollen is concealed. It is assumed that bee-pollination was impotent in the divergence of papilionoid flower in this species. The flower of this species, as it was mentioned before, has nectar guide and lading platform, suitable for pollinators' visitation. When a flower is visiting by a pollinator, the keel is moved downwards, the stigma and the anthers are exposed at the tip of the keel, the keel moving back to the initial position after the pollinator has left the flower, this finding is supported by Endress (1994).

The inclusion of the pollination organs in the keel has led to a particular differentiation of the stigma. Although many are self-compatible, pollen which is normally deposited on the stigma before anthesis cannot germinate unless tripping at the anthesis is caused by a visitor. The stigma is covered by a membrane, which prevents pollen germination. Only by tripping process is the membrane disrupted, allowing the pollen, now in closer contact with the wet, papillate stigma surface, to germinate (Endress, 1994). In addition, Riano et al. (1999) found that germination of pollen grains of the two in family Fabaceae occurs only after rupture the stigmatic surface.

As it was mentioned before, nectar sugar concentration and composition related to pollinator species. Overall nectar production agrees with a previous study (Riano et al., 1999; Sampson and Cane, 1999), Nectar sugar concentration was agreed with a study of Riano et al. (1999), Langenberger and Davis (2002), this value is higher than in plant that has both nocturnal and diurnal visitors (Slauson, 2000), and bird-pollinated species (Yumoto, 2000). The sugars identified were informed that there was sucrose dominant, Bernardello et al. (2000) founded that; there were higher sucrose proportion in the species pollinated by hummingbirds. This therefore confirms by the suggestion of Endress (1994) that bee-flowers are sucrose-rich, and hummingbird-flowers are often derived from bee-flowers. The result of this study also support the finding that *A. sericea* should be bee-pollinated species.

1. Behavioral Features of the Visitor and Pollinator-Plant Interaction

In A. sericea visitors approach flowers for four reasons, pollen, nectar, sepal and leaf; and directly associated with each other. The visitors involved are grouped into pollinators and non-pollinators.

1.1 Pollinators

From the present observation *A. Sericea* has at least twenty different flower visitors. Some of them are classified as its pollinators due to their foraging behaviors that lead some contacts between anthers and stigma or transferring of pollen from anthers to stigma in one way or another. The bees in the genus *Megachile* seem to be the most significant pollinator, especially *M. velutina* since it could be found in every studied sites that have fruit setting. *M. monticola* may be also an important pollinator but it found only in some studied sites. The rest of the Megachile have also potential to be pollinators according to their behaviors, but are clearly less important, at least in the present studied sites, as their appearances are distinctly rare. In addition the report of *Megachile* as a pollinator of fabaceous plants was previously mentioned in *Hedysarum boreale* (McGuire, 1993) and four species of 2 family: Leguminosae and Xanthophyllaceae in which the flowering times was synchrony with the emergence of *Megachile* (Momose et al, 1998).

The behavior of *Nomia* spp. and also *Pithis smaragdula* suggest their potential to be effective pollinators too, but they are not commonly found like *M. velutina.* However, *Nomia* is the excellent pollinators for several other species, as well as pea (Free, 1970; Proctor, Yeo and Lack, 1996). *Pithis smaragdula* sometimes behaves like nectar thief as it sucks the nectar from the flower but takes no part in pollination.

1.2 Non pollinator

Many investigations suggested that *Xylocopa* is a common pollinator in some large-flowered Fabaceae like *Centrosperma*, *Canavalia*, *Vigna* and *Harpalyce* (Endress, 1994: Momose et al, 1998; Mack and Milligan, 1998). *Xylocapa* is found to be a common visitor in *A. sericea* too, but it could not pollinate the flower. Although many floral characters of *A. sericea* are like Xylocopa-flower, i.e. strong architecture, hidden nectar, unsaturated color, one-day flower and steady-state flowering strategy (Endress, 1994) but it seems that the pedicel of *A. sericea* is not strong enough, compared with other fabaceous genera which pollinated by *Xylocopa*, to directly support the weight of such a large bee. Thus, *Xylocopa* has never visited the flower of *A. sericea* directly on the wings and keel so it has less chance to touch the male and female parts of the flowers which locate at the tip of the keel.

After *Megachile*, *Nomia*, *Pithis* and other visitors left the flower, there will be a small amount of pollen deposited on the surface of petal. Stingless bees (*Trigona* sp) and beetles are found to collect those pollen grains but they do usually not touch stigmas.

The behavior of the oil beetles (*Mylabris phale*rata) is quite interesting. Since they always bite parts of flowers then the pollinating stigma may be harm by their behavior too. One might suggest that the low percentage of fruit setting at Sakaerat Environmental Research Station may be resulted in one way from the damaged stigmas and lead to unsuccessful pollen germination. However, the percentage of flower that damaged by the activity of oil beetles is very low (less than 1 %) thus, the main reason of low fruit ought not to deal with this beetles.

The appearance of purple throated-sunbird (*Nectarinia sperata*) as a flower visitor of *A. sericea* is not surprising. Even though the majority of bird-flowers often has a relatively long and tubular part with protruding stamens and stigmas (Endress, 1994), but the basic structure of bird-pollinated plant can be diverse as a wide range of plant families is involved in bird-pollination and a number of species of *Erythrina* (Fabaceae) is found to be pollinated by birds too (Bruneau, 1997; Proctor, Yeo and Lack, 1996). However, it is quite clear in *A. sericea* that this sunbird has no role in pollination. They just come for the nectar only and their appearance only on the rainy days may suggest that they are actually not attracted by the flower of *A. sericea* in normal situation.

It can conclude that, there have plant/animal coadaptations in this study. Theses are certainly mutual adaptations, often complex and indispensable (Abrahamson, 1989; Jolivet, 1992, 1998), particularly in the case of *Megachile* ssp., this genus was found pollinated *A. sericea* all study sites.

Fruit Setting Investigations

1. Pollinator-Exclusion Experiment

A pollinator-exclusion experiment was conducted to determine whether insect pollinators were necessary for fruit and seed production. This experiment proved of special importance because of the deposition site of the pollen due to flower morphology. The pollen can deposit on the stigma head during anthesis. The stigma becomes receptive simultaneously with anther dehiscence time, which theoretically could result in self-fertilization (autogamy). The results of the bagging experiments, in addition with floral morphology, therefore indicated that pollinators are necessary for pollination and fertilization in this species. Fruit set was absolutely reduced when insect pollinators were excluded from inflorescence, no fruit was formed in the absence of pollinators. Kaye (1999), Vaz et al. (1998), and found that insects capable of tripping the floral mechanism were present and can enhance fruit set in Fabaceae. In this study, none of 30 bagged inflorescences produced pods. Insects capable of tripping the floral mechanism were presence at all study sites. It can be concluded that pollinators conducted an importance role in pod setting in *A. sericea.*

2. Fruit Setting Related to Plants Distance

In this study, inflorescences with pod and average pod numbers in inflorescence in plants which has the nearest others within 20 meters were higher than in farther 3 plants, which the nearest others was 300 meters. This data may be resulted from different in probability of pollen to transform from one plant to each other. Pollinators make a significant contribution to production in plant by their mechanical action on flowers, thus promoting self-pollination and also effecting cross-pollination (Kittelson and Maron, 2000). As it was mentioned in Dibble and Drummond (1997), shorter distances between flowers may allow bees to forage with less effort, and could contribute to any type of pollinations. In an outcrossing plant such as *A. sericea*, the likelihood of crossed mating increases with the number near plants. If distance between plants (density) and plant abundance are factors in attraction of bees to flowers, then this study appears to support optimal foraging theory (Dibble and Drummond, 1997 and papers cited therein). Foraging effort is reduced when flowers are closer together.

3. Percentage Fruit Set, Percentage Seed Set, and Average Seed Weight

From the investigation at Sakaerat Environmental Research Station in 2000 and 2001, percent of infructescence set were only slightly difference, however, percent of pod set and percent of seed set were almost no difference at all. Comparing with the data from *ex situ* study sites in Pharphayom District Phatthalung Province in 2002, it was found that, percent of infructescence set, percent of pod set and percent of seed set were higher. Wagner (2000) investigated *Acacia constricta* and found that, although 15% of inflorescence setting pod but average pod/infructescence was low in both outcrossed and selfed experiment respectively, in addition Kaye (1999) studied in *Astragalus australis* also reported the low fruits and seeds set, it can be concluded that fruit setting rate in Fabaceae are low. In agreement with the observations of Vaz et al. (1998) in cowpea, in this species fruit set following self-pollinations and cross-pollination were higher than in other studies. In the present study it was found that, within fruits, seed per pod is always two and fruit with lack of fertilization or seed abortion almost not found. There also no difference on average seed weight form 3 samples, as it was mentioned in the study of Kaye (1999), that there was no treatment effect on seed set per fruit and seed weight. In contrast, Arathi et al. (1999), studied in *Pongamia pinnata*, only one of two ovules develops into a seed in most of the pods, in addition with Helenurm and Schaal (1996) study in *Lupinus texensis* and found that seed abortion in this species was 17-28%.

From the present study, it was found that stigma with germinating pollen in opened pollinated flower in natural habitat usually more than 30%, these results together with the fact that fruit are formed with clearly enlarged ovules relative to the virgin ovules after opened pollination performed, indicate that fertilization occurs. However pod setting rate was only about 0.1% in natural habitat and 0.3% in study site in Phuttalung, it mean only about 1% of pistil with germinated pollen can develop to be young pod. In addition with the fact that, about 10% of young pod cannot continue develop to be mature pods. As in previous studies, (Barrett et al., 1996; Riano et al. (1999) pointed out that, this suggests the existences of a postzygotic rejection mechanism, which could be due either to the existence of late-acting self-incompatibility; those conditions where the functioning of self-incompatibility located within the ovary, either prior to fertilization, or as the result of the abortion of the selfed ovule or fruit (Procter et al.1996; Richards, 1997); or to an early action of inbreeding depression, although there are lines of evidence that seem to point to the second possibility. In contrast with the study of Sage et al. (1999), in that study the results indicated that self-sterility can operates prezygotically but does not involve differential pollen tube growth typical of many self-incompatibility systems. The results of the present study offer support for the belief that late-acting was performed in this species.