CHAPTER 1

General Introduction and Thesis Outline

Reproductive success of insects mainly presupposes the effective location of mates and oviposition sites as well as the avoidance of natural enemies and unsuitable abiotic conditions (Dicke and Grostal, 2001). Thus, organisms are assumed to be well adapted to gather information on the profitability and the risks of their environment. Apart from acoustic, tactile and visual stimuli, particularly chemical cues play a vitally important role in the searching process of most, if not all, insects (van Alphen and Jervis, 1996; Wyatt, 2003).

Terminology of infochemicals

Information conveying compounds, so-called infochemicals, are divided into two broad categories according to their use within and between species (Fig. 1) (Nordlund and Lewis, 1976; Dicke and Sabelis, 1988). Pheromones are substances that mediate interactions of individuals belonging to the same species. Most of them operate as releasers by causing immediate behavioural changes in the receiver (Wilson and Bossert, 1963). In contrast, primer pheromones induce physiological changes in the receiver, such as sexual maturation. However, some pheromones are known to have both a primer and releaser function (e.g, the honeybee queen pheromone) (Wyatt, 2003). According to their function, pheromones are subdivided into, e.g., territory marking pheromones, alarm pheromones, trail marking pheromones, aggregation pheromones and sex pheromones (Nordlund, 1981). Latter ones are widespread among insects released by males or females to induce behavioural responses directly or indirectly leading to mating (Shorey, 1973; Powell, 1999). Allelochemicals, the second group of infochemicals, mediate interactions of individuals belonging to different species. Depending on the benefits for sender and receiver, they are categorised into allomones (beneficial for the sender), kairomones (beneficial for the receiver) and synomones (beneficial for sender and receiver) (Nordlund and Lewis, 1976; Dicke and Sabelis, 1988). However, a given chemical can have several biological functions within a complex
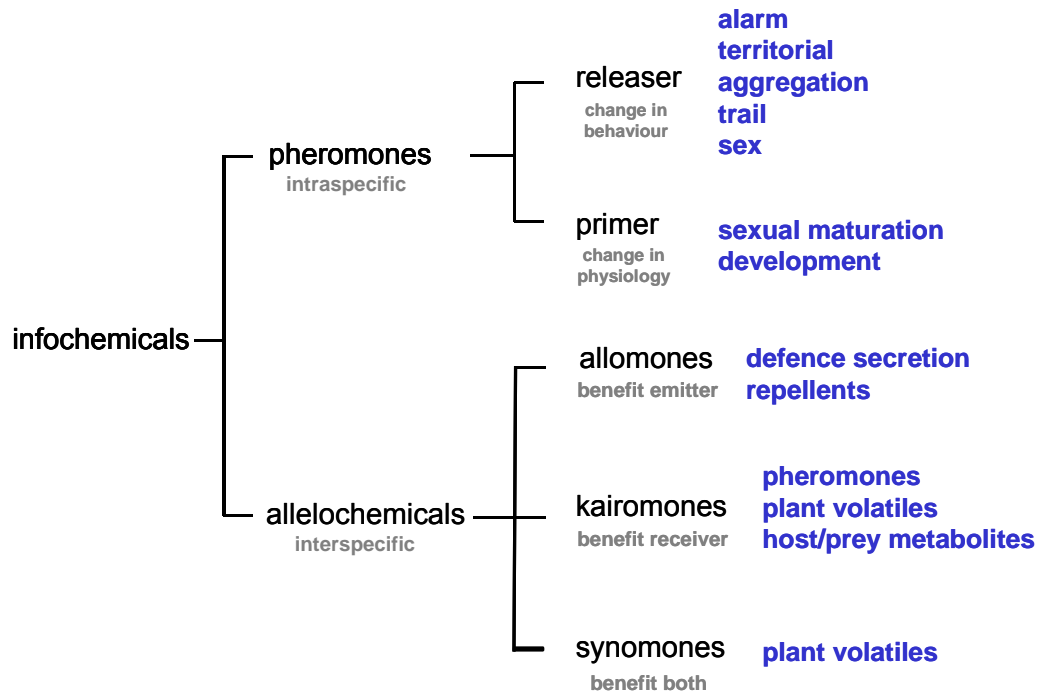
network of interactions and thus, the classification of infochemicals can rapidly become complicated. For example, sex pheromones of insects are often exploited by their natural enemies as foraging kairomones (Powell, 1999; Steidle and van Loon, 2003).

Chemoecology

Chemical ecology is a scientific discipline that explores ecological and evolutionary processes in which infochemicals play a crucial role (Vet, 1999). Major subjects of this relative new research field are the elucidation of both the chemical structures and the ecological functions of the mediating substances (Takken and Dicke, 2006). Recent improvements of analytical techniques (especially in chromatography) have drastically simplified and quickened chemoecological research. However, many infochemicals, especially pheromones, have been found to consist of multiple components acting synergistically when combined in a particular ratio (Wyatt, 2003). Thus, the complexity of chemical cues and signals involved in the communication of insects still pose an enormous challenge for every chemoecologist. In addition, the mere characterisation of the chemical blend released by an organism rarely results in determination of the initiating chemicals. Behavioural as well as neurophysiological experiments are required to finally establish the biological function of single components. However, as infochemicals of pest insects and their natural enemies are better understood, they provide sustainable methods of pest control as alternatives to the exclusive application of broad-spectrum insecticides (Burkholder, 1985; Lewis and Martin, 1990; Phillips, 1997).

Reproductive biology of parasitoids

Parasitic Hymenoptera, mainly embracing the Ichneumonoidea, Chalcidoidea, Cynipoidea and Proctotrupoidea, are one of the largest and most diverse groups of insects with many species playing regulative roles both in agricultural and natural ecosystems (Godfray and Cook, 1997; Quicke, 1997). By definition, they are carnivorous insects that develop by feeding on (ectoparasitoids) or in (endoparasitoids) various life stages of other arthropods (mainly insects), finally resulting in the death of the host. Though parasitoidism is known in several other

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                                                    alarm
                                     releaser        territorial
                                     change in       aggregation
                                     behaviour       trail
                    pheromones                       sex
                    intraspecific
                                     primer          sexual maturation
                                     change in       development
                                     physiology
infochemicals
                                     allomones       defence secretion
                                     benefit emitter repellents

                                                     pheromones
                    allelochemicals  kairomones      plant volatiles
                    interspecific    benefit receiver host/prey metabolites

                                     synomones       plant volatiles
                                     benefit both
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Fig. 1 Terminology of infochemicals after Dicke and Sabelis (1988), with examples.

insect orders, e.g., in Diptera, Coleoptera or Lepidoptera, the term "parasitoid" used in this thesis is exclusively restricted to hymenopteran species.

Mating systems of parasitoids are predominantly influenced by the spatial distribution of their hosts. In gregarious[1] and quasi-gregarious[2] species, mating commonly occurs at the emergence site (Godfray, 1994). Males are often protandrous, i.e., they emerge before females and wait at this site for females to copulate. In species where both sexes emerge spatially separated, males often localise female emergence, oviposition or feeding sites by using infochemicals for long-range orientation (Godfray and Cook, 1997).

The majority of female parasitoids seems to be monandrous, i.e., they mate only once in their lifetime (Gordh and DeBach, 1978; Ridley, 1993), although there is much variation among the species (van den Assem, 1986). Sperm received from a single insemination is often sufficient to fertilise a lifetime supply of eggs (Thornhill and Alcock, 1983). In addition, females benefit also from producing male offspring

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[1] Gregarious species lay several eggs per host.
[2] According to Godfray (1994), solitary parasitoids (laying one egg per host) are termed quasi-gregarious when they parasitise hosts occurring in aggregations.

without mating due to arrhenotocous parthenogenesis. Thus, the reproductive success of females is primarily restricted to the entire number of eggs they lay as well as the survival and fecundity of their offspring. By contrast, male fitness is predominantly related to their ability of finding mates and fertilising more eggs than other males in the population. Competition for mates has evolved various male mating tactics including deception to increase the access to receptive females or ensure greater paternity success (Ayasse et al., 2001). Intraspecific sexual mimicry, i.e., chemical, behavioural or morphological imitation of the opposite sex for sexual purposes, appears to be relatively common among insects and was also previously demonstrated in a parasitic wasp species (Field and Keller, 1993).

Sex pheromones of parasitoids

Although sex pheromones are assumed to play a crucial role in the mate finding process of most parasitic Hymenoptera, they have been rarely investigated (Kainoh, 1999). The existence of these bioactive compounds has been demonstrated in several studies, very few of these, however, have been chemically characterised or even identified (Kainoh, 1999; Keeling et al., 2004). To date, no more than nine sex pheromones of parasitic hymenopterans have been reported, most of them from larger species of the Ichneumonoidea (Table 1). One reason for this lack of knowledge is probably the fact that the biologically active substances occur in incredibly minute amounts, often obscured by inactive major components (Quicke, 1997; Keeling et al., 2004). Another problem may arise from the limited knowledge about the life history of many parasitoid species. Thus, it remains difficult to establish parasitoid cultures in the laboratory providing enough material for extensive pheromone studies. Despite these problems, however, the interest in parasitoid sex pheromones is increasing, particularly due to their practical value in integrated pest management. Sex pheromones might be used for monitoring parasitoid populations in the field and enhance their impact on insect pest populations (Lewis et al., 1971; Powell, 1986; Brodeur and McNeil, 1994).

Male parasitoids often use volatile sex pheromones released by the females to locate their mates over long distances (Kainoh, 1999). Attraction of males to female volatiles was shown by using field traps containing females or extracts of females (Lewis et al., 1971; Eller et al., 1984; Swedenborg and Jones, 1992a; McNeil and Brodeur, 1995) as well as wind tunnel and olfactometer experiments (Cole, 1970;

Powell and Zhang, 1983; McAuslane et al., 1990). In some cases, pheromones elicit not only responses in individuals of the opposite sex but also in those of the same sex. Pheromone-baited traps containing females of the braconid *Cardiochiles nigriceps* attracted males and other females, although the relevance of female behaviour was not fully understood (Lewis et al., 1971). In other cases, both sexes produce the same sex pheromone, at least for a short period. In *Itoplectis conquisitor* freshly emerged males and females are attractive to older males (Robacker et al., 1976). However, pheromone activity of the males decreased soon after emergence. Male-produced sex pheromones of high volatility seem to be rare throughout the parasitic Hymenoptera (Gordh and DeBach, 1978). Very few studies show evidence for the presence of male-derived attractants by behavioural experiments. Gonzalez et al. (1985) reported a male pheromone that strongly attracts unmated females of *Melittobia australica* and *M. femorata*. However, nothing is known about male pheromone chemistry to date.

In addition to attracting potential mates over long distances, sex pheromones are also involved in the mating behaviour (i.e., courtship and copulation) of parasitic wasps. The involved chemicals are mostly stable and of low volatility and thus, recognised by contact or within a few millimetres. Several studies provide evidence for female-derived sex pheromones that stimulate arrestment and courtship behaviour in male parasitoids (Yoshida, 1978; Simser and Coppel, 1980; Mohamed and Coppel, 1987; Shu and Jones, 1993; Syvertsen et al., 1995; Sullivan, 2002). Ethological aspects of the courtship behaviour in parasitic Hymenoptera have been the subject of intense research (Godfray, 1994; van den Assem, 1986; Quicke, 1997). Some of the most detailed studies examined species of the taxon Chalcidoidea (e.g., Barras, 1960, 1961; van den Assem, 1970; van den Assem and Povel, 1973; van den Assem, 1986; van den Assem and Werren, 1994). When encountering receptive females, males commonly perform a series of well-defined stereotypical behavioural elements such as wing vibration, mounting and antennal movements. Male pheromones (aphrodisiacs) may be involved in the mating process of parasitic wasps by inducing female receptivity (van den Assem et al., 1980b, 1981).

Scarcely anything is known about the site of pheromone production in parasitic Hymenoptera. Pheromone activity was localised in the abdomen (Tagawa, 1977; Yoshida, 1978), thorax and/or head (Takahashi and Sugai, 1982; Kainoh and Oishi,

1993) or in all parts of the body (Ruther et al., 2000; Sullivan, 2002). The latter case suggests that several pheromone glands are uniformly distributed over the cuticle surface or that bioactive chemicals have their origin in a gland and are spread across the entire body by diffusion or insect cleaning behaviour. In several studies, the Duffour's gland is supposed to be involved in pheromone production (Vinson, 1978; Simser and Coppel, 1980; Syversten et al., 1995) and it is likely that this gland is of general importance in many parasitoid species (Quicke, 1997). However, other studies reported the location of the sex pheromone gland at the base of the second valvifer of the ovipositor system (Tagawa, 1977, 1983) or in the tibia of the hindlegs (Kainoh and Oishi, 1993).

Table 1 Identified sex pheromones in parasitic Hymenoptera.

Taxon	Activity	Compound(s)	References
Ichneumonoidea			
Braconidae			
Ascogaster reticulates	short-range	(*Z*)-9-hexadecenal	Kainoh et al. (1991)
Ascogaster quadridentata	long-range	(*Z,Z*)-9-12-octadecadienal	DeLury et al. (1999)
Macrocentrus grandii	long-range	(*Z*)-4-tridecenal; (3*R*,5*S*,6*R*)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one	Swedenborg and Jones (1992a,b) Swedenborg et al. (1993) Swedenborg et al. (1994)
Cardiochiles nigriceps	short-range	(*Z,Z*)-7,13-heptacosadiene and at least one other alkadiene in combination with hydrocarbons	Syvertsen et al. (1995)
Ichneumonidae			
Eriborus terebrans	short-range	polar component(s) and hydrocarbon(s) as synergist	Shu and Jones (1993)
Syndipnus rubiginosus	long-range	ethyl (*Z*)-9-hexadecanoate	Eller et al. (1984)
Exetastes cinctipes	long-range	(*Z*)-8-dodecenyl acetate; (*Z*)-11-tetradecenyl acetate (possible sex pheromone)	Hrdy and Sedivy (1979)
Itoplectis conquisitor	long-range	neral and geranial as compounds of the sex pheromone system	Robacker and Hendry (1977)
Cynipoidea			
Charipidae			
Alloxysta victrix	long-range	6-methyl-5-heptene-2-one	Micha et al. (1993) Höller et al (1994)

Host-associated kairomones

Apart from pheromones, also kairomones play a crucial role in the chemical communication of insects and may have highly diverse functions for the receiving organisms (Ruther and Steidle, 2002). In parasitic Hymenoptera, kairomones are mainly involved in the host selection process of females including the habitat preferences and a series of consecutive behaviours that lead to the successful parasitisation of the host (Vinson, 1998). While volatile substances primarily enable the location of the host habitat and the host within the habitat, less volatile compounds are used by the parasitoids to recognise and finally accept the host (Godfray, 1994; Quicke, 1997). These chemicals may originate from the host itself (e.g., cuticular compounds), its products (e.g., silk, faeces) or from organisms that live associatively with the host (e.g., bacteria, fungi, mites) (Vet and Dicke, 1992; Powell, 1999; Steidle and van Loon, 2002). In addition, parasitoids rely also on volatiles of the host's food plant (synomones) induced by feeding or oviposition of the herbivore (Tumlinson et al., 1993; Turlings and Wäckers, 2004; Hilker and Meiners, 2006). The response to chemical cues in the context of foraging may change during the parasitoid's life as a result of associative learning (Turlings et al., 1993; Vet et al., 1995). In contrast, the behavioural response to sex pheromones is commonly determined by genetic and physiological factors.

While the host location behaviour of parasitic Hymenoptera and the mediating chemicals have been intensively investigated (Vinson, 1976, 1984; Nordlund et al., 1988; Vet and Dicke, 1992; Godfray, 1994; Quicke, 1997), there are relative few studies dealing with the aspect of host habitat preference (Vinson, 1998). However, the accurate assessment of the host habitat and host suitability for offspring development is crucial since females are supposed to maximise their reproductive success by ovipositing on high-quality hosts (Godfray, 1994). Hence, habitat preferences of parasitoids are determined by a number of internal (e.g., age, hunger, experience) and external (e.g., host availability, inter- and intraspecific competition, risk of predation or diseases) influences as well as environmental factors (e.g., temperature, humidity, wind, light) (Vinson, 1998).

In a few papers, however, host-associated kairomones have been shown to influence also the sexual communication of parasitoids (Matthews et al., 1979; Ruther and Steidle, 2000). By using the same chemical cues for mate finding as

females use for host finding, males may increase the probability of sexual encounters at potential oviposition sites. This male strategy might be of particular importance in parasitoid species where females do not produce a long-range sex pheromone to attract males.

Study organisms

The parasitoid species investigated in this thesis belong to the large taxon Pteromalidae (Chalcidoidea) including more than 3500 species of morphologically and biologically diverse habits. There are several features that make pteromalids excellent study organisms for understanding chemically mediated mate and host finding in parasitoids. Firstly, they exhibit life histories that are widespread among the parasitic Hymenoptera and thus findings on the Pteromalidae might be also transferable to other parasitoid taxa. Secondly, basic and applied research on mate and host finding of pteromalids has been already done. Especially the well-defined courtship behaviour and the mating systems of this family are better understood than in many other taxa of parasitic Hymenoptera (van den Assem, 1986). Finally, many species of the Pteromalidae are very easy to work with in the laboratory. Short generation time, high offspring production and often commercially available hosts make them particularly suitable for mass rearing and thus, for extensive chemical analyses and behavioural experiments.

Most studies presented in this thesis were performed with *Lariophagus distinguendus* (Förster), a solitary ectoparasitoid attacking larvae and pupae of several stored product infesting beetles that develop in grains and plant seeds (Steidle and Schöller, 1997). Since this parasitic wasp is markedly polyphagous, it is considered as a potential candidate for biocontrol in stored product protection (Steidle, 1998). Host location behaviour of *L. distinguendus* has been extensively examined during the past years (Steidle and Schöller, 1997; Steidle, 2000; Steidle and Fischer, 2000; Steidle and Ruther, 2000; Steidle et al., 2001). However, hardly anything is known about the infochemicals involved in the sexual communication of this species. The courtship behaviour of males has been demonstrated to be elicited by a female-derived sex pheromone that has only an activity range of a few millimetres and is most probably produced in the abdomen of the parasitoids (Ruther et al., 2000). In contrast, volatile pheromones mediating the mate finding of *L. distinguendus* over long distances were not found (Ruther and Steidle, 2000).

Another pteromalid species investigated in this thesis is *Nasonia vitripennis* (Walker), a model organism for the study of parasitic Hymenoptera biology. It parasitises pupae of numerous cyclorraphous fly species. Larvae of *N. vitripennis* develop not singly but gregariously within the host puparium (an outer shell around the fly pupa). Although this pteromalid species has been the subject of numerous studies investigating courtship behaviour (e.g., van den Assem and Vernel, 1979; van den Assem et al., 1980b; Jachmann and van den Assem, 1996) and mating characteristics (e.g., van den Assem and Visser, 1976; van den Assem and Feuth de Bruijn, 1977; van den Assem et al., 1980a), the chemical elucidation of female and male sex pheromones involved has received no consideration so far. It is known that a female-derived sex pheromone stimulates the courtship behaviour in males who in turn induce the readiness of females to copulate by releasing an aphrodisiac from their mandibular gland (van den Assem et al., 1980b). Moreover, males of *N. vitripennis* are assumed to mark their territories by chemicals that are attractive for both females and other males.

Thesis outline

The main goal of this thesis was the structural and functional characterisation of infochemicals operating as pheromones and host-associated kairomones in mate finding and recognition of Pteromalidae. Since the identification of natural compounds used by parasitoids for sexual purposes represents a nearly unexplored field of chemical ecology, findings of this work provide new insights into the mechanisms enabling the sexual communication of these ecologically and economically important insects. A further aim was the evaluation of the potential role of host-associated kairomones in the host habitat assessment of *L. distinguendus*, a so far neglected aspect that should round off the already wide knowledge about the host selection process of this pteromalid species. More specifically, the present thesis aims to answer the questions of the following aspects:

Chemical characterisation of the courtship pheromone in *L. distinguendus:* What is the chemical nature of the bioactive compounds and when do parasitoids start to produce them?

Comparative study on the pheromone chemistry in *N. vitripennis*: Are the findings of the preceding study on *L. distinguendus* transferable to the related pteromalid species *N. vitripennis*? What are the differences?

Characterisation of male-specific compounds in *N. vitripennis*: Do males use these compounds as a sex pheromone to attract the females? And if so, what is the chemical structure?

Mating characteristics and reproductive performance of *L. distinguendus*: What is the mating frequency of males? Does the male mating status affect the tendency of females to mate a second time with respect to the transferred sperm?

Long-range orientation of male *L. distinguendus* during mate finding: Do males rely on the same host-related kairomones that females use for host finding to increase the probability of matings?

The role of host-associated kairomones in host habitat preferences of *L. distinguendus*: Are females able to avoid negative fitness consequences by using host-related odours during host habitat assessment?

In ***chapter 2*** the courtship pheromone of *L. distinguendus* was chemically characterised by GC-MS[3] analyses of whole body extracts and assaying fractions of different polarities for behavioural activity. Interestingly, it turned out that both female and male *L. distinguendus* produce the sex pheromone during pupal development but in males the chemicals wear off soon after emergence. Further experiments were conducted to investigate whether males searching for mates are able to recognise grains containing parasitoids shortly before emergence and whether they are able to differentiate between the sexes. Finally, it was discussed whether the phenomenon of immature males having the female courtship pheromone might be beneficial and thus, would be a novel case of pre-emergence chemical mimicry.

Based on the results of the preceding chapter, the putative function of hydrocarbons as components of the courtship pheromone of *L. distinguendus* was studied more thoroughly in ***chapter 3***. Hexane fractions of different male and

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[3] coupled gas chromatography-mass spectrometry

female life stages were tested for behavioural activity and analysed by GC-MS. Qualitative and quantitative differences of bioactive and -inactive hydrocarbon profiles were compared by multivariate statistical methods to narrow down candidate chemicals involved in pheromone activity.

Studies of mating characteristics and reproductive performance of *L. distinguendus* are presented in ***chapter 4***. Basic data such as mating potential, offspring production and sex ratio were acquired. Moreover, it was investigated whether females that had mated with sperm-depleted males tend to mate a second time to replenish their sperm supply and whether therefore, they maintain a higher pheromone activity. The results of this chapter were discussed with respect to a putative mating strategy of sperm-depleted males in *L. distinguendus*.

To study whether the use of cuticular hydrocarbons as courtship pheromones is a common feature in pteromalids, ***chapter 5*** presents a comparative study employing *N. vitripennis*. Compounds mediating the male courtship behaviour were characterised by elucidating the polarity, range of activity and longevity. Furthermore, it was tested whether the pheromone is detectable already in pupae of either sex as demonstrated before in *L. distinguendus*. Behaviourally active fractions of female extracts were analysed by GC-MS and statistically compared with inactive fractions from males. Finally, the potential role of tactile or visual stimuli in the courtship behaviour of *N. vitripennis* males was examined.

The identification of the first male sex pheromone in parasitic Hymenoptera is reported in ***chapter 6***. After determining the structure of the male-specific compounds in extracts of *N. vitripennis*, it was tested whether the mixture attracted both naïve female and male parasitoids and whether the mating status affects the female response to the male-derived chemicals. Moreover, the age dependency of pheromone titers, the possible site of biosynthesis and release characteristics of individual males were examined.

Since volatile sex pheromones are missing in *L. distinguendus*, other infochemicals than pheromones have to be considered to mediate the long-range orientation by males towards females. Thus, the study described in ***chapter 7*** addresses the question whether males innately rely on the same host-associated volatiles for mate finding as females use for host finding. The response of naïve parasitoids to larval

faeces of the host, headspace extracts of larval faeces, as well as fractions of these extracts were tested. Active fractions were analysed to elucidate the chemical composition of the host-associated kairomones.

The study presented in *chapter 8* deals with the potential role of fungal volatiles as indicators for suboptimal host patch quality. It was investigated whether the long-range orientation, host recognition behaviour and reproductive success of *L. distinguendus* females is influenced by mould infestation of the host cultures. A common fungal metabolite occurring as major volatile in moulded host cultures was tested with respect to its repellent effect.

In *chapter 9* the main results and conclusions of this thesis are discussed in a broader chemoecological context. A complex and fascinating network of infochemicals mediating the mate and host finding processes of *L. distinguendus* is presented by combining the results of this work with those of previously published studies. Finally, directions for future studies are provided.

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