Red Imported Fire Ant (*Solenopsis invicta*):
A review of the literature regarding the determination of colony age

A report prepared for MAF Biosecurity New Zealand

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Summary

MAF Biosecurity New Zealand (MAFBNZ) is currently responding to a third incursion of the red imported fire ant (RIFA) (*Solenopsis invicta*). MAFBNZ's RIFA Technical Advisory Group has identified several areas of knowledge essential for effective response to incursions, one of which is the ability to determine colony age. Colony age is valuable for determining the spatial extent required for surveillance and treatment by estimating whether the colony has produced a nuptial flight. This report aims to critically synthesise the international literature contained within peer-reviewed journals and technical reports on the methods available for determining the age of RIFA colonies. Five methods are suggested by the literature: colony size, mean worker head width, caste proportions by mean head width, caste proportions by worker weight and worker fat content. In general, reporting was poor regarding aspects such as social form and environmental conditions prevalent upon colonies. Research has been restricted to the USA. It is recommended that RIFA colonies be assessed using the colony size, mean worker head width and caste proportion methodologies together to maximise reliability. Tschinkel's (1988) definitions of worker caste should be favoured where possible. Future research should include factors known to confound assessment techniques, with particular reference to temperature and the applicability of confounding factors within New Zealand.

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1. Introduction

1.1 Rationale

MAF Biosecurity New Zealand (MAFBNZ) is currently responding to a third incursion of the red imported fire ant (RIFA) (*Solenopsis invicta*). In June 2006, a nest was found at Whirinaki, New Zealand. Although this incursion appears to have been eradicated and will be declared so after one more year of monitoring, the difficulties associated with detecting fire ants at the New Zealand border makes future incursions likely.

Where it has invaded the southern states of America, *S invicta* has become a significant suburban and agricultural pest (Tschinkel, 1998). New Zealand is predicted to be climatically suitable for RIFA (CLIMEX, 2001), making biosecurity action essential. MAFBNZ wishes to improve its ability to respond to incursions in the future.

MAFBNZ's RIFA Technical Advisory Group has identified several areas of knowledge essential for effective incursion responses. One of these areas is the ability to determine colony age. Colony age is valuable for assessing whether the colony has sent out a nuptial flight, and therefore will determine the spatial extent of surveillance and treatment. It also aids in identifying potential pathways of entry and spread, based upon how long the colony has been present at the site.

1.2 Background

Two life history strategies are known amongst RIFA colonies: monogyne (containing a single queen) and polygyne (containing multiple queens within a colony). The basis of the difference between these two social types is allelic heterogeneity at the Gp9 locus. Monogynous queens exclusively carry the Gp-9^{BB} genotype. Pre-reproductive polygyne queens carry Gp-9^{BB}, Gp-9^{Bb} or Gp9-^{bb}, but reproductive queens are almost exclusively Gp-9^{Bb} (Goodisman, DeHeer, & Ross, 2000).

Distinct differences in reproductive strategies exist between the different social forms. Pre-reproductive monogyne queens (alates) undertake nuptial flights, mating in the air and potentially travelling large distances before landing to found a new colony (Goodisman et al., 2000; Tschinkel, 1998). Monogyne queens typically dig claustral chambers in which they raise the first generation of workers (minims, or nantic workers) alone (Tschinkel, 1998). In some cases, queens may aggregate and co-found, but workers kill all but one queen after founding (Tschinkel, 1998; Vargo & Fletcher, 1986b). Polygyne queens demonstrate other reproductive techniques in addition to nuptial flights. They frequently mate in the

nest and disperse only short distances from their natal nest (Goodisman et al., 2000; Tschinkel, 1998). Polygyne queens are thought to rarely attain the body reserves necessary for independent founding and more commonly take workers with them in the founding of new colonies, a process known as 'budding'(Tschinkel, 1998).

Given the short distances involved in budding colony reproduction, nuptial flight reproduction is considered a greater concern for biosecurity as new colonies will be more difficult to detect. Therefore, in considering the relationship between colony age and the onset of reproduction, this report considers only time to alate production.

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2. Objectives

The report aims to critically synthesise the international literature on the methods available for determining the age of RIFA colonies.

The report will make recommendations to Biosecurity New Zealand, as to knowledge gaps in this area.

This report reviews articles from peer-reviewed journals, and technical reports. It does not review websites, or articles from non-peer reviewed journals.

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3. Methods

Information was obtained by searching computer databases (CAB abstracts, Current Contents, Agricola, Biological Abstracts) for relevant scientific papers and technical reports; cross-referencing from these publications; and querying international ant researchers and biosecurity practitioners.
Information gathered was:
- Methods of determining the age of a RIFA colony.
- Relationship between colony age and reproduction via nuptial flights

Information was sought from the following experts:
- Dr. Walter R. Tschinkel, Professor of Biological Science, Florida State University, Florida, USA.

- Dr. Sanford D. Porter, Research Entomologist, United States Department of Agriculture, USA
- Dr Charles Barr, Extension Program Specialist, Texas A & M University, Texas, USA
- Dr Shaun Forgie, Researcher, Biodiversity and Conservation, Landcare Research Ltd., New Zealand
- A general request for information was sent to the Aliens-L biosecurity listserver

4. Results

4.1 Colony Size

A logistic curve (N= 220,000 / (1 + 83 e ^ 1.26)) was found to best explain field colonies from Florida between five months and 12 years old (of unspecified social form) (Figure 1). The logistic model was a poor fit for both field and lab-raised colonies younger than 5 months, but no alternative model was fitted to this data (Tschinkel, 1988). In the case of several older colonies, estimates of colony age contain error of up to three months. The author predicts colony sizes to be 10-15% underestimated because foraging workers were not included. However, there is some evidence that the proportion of forgers decreases with colony size (Sorensen, Mirenda, & Vinson, 1981), making estimates difficult.
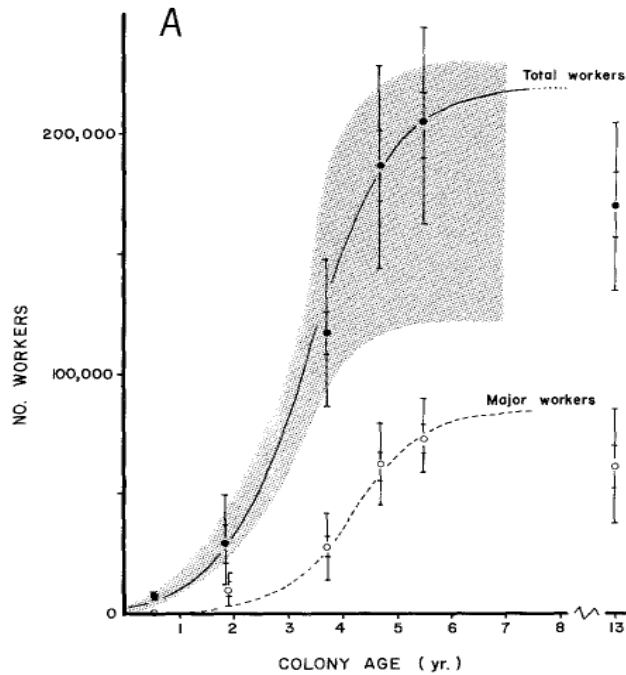
Figure 1: Growth of *Solenopsis invicta* colonies. Curves are the best-fit logistic function. The shaded area shows the limits of seasonal variation of mean colony size. Error bars show S.D. and S.E.M. Adapted from (Tschinkel, 1988).

The logistic curve data was combined with that of Markin et al. (1973) and unpublished data from Sanford Porter to estimate seasonal variation in colony growth (Tschinkel, 1993a) (Figure 2). The model is described by: $N = (165{,}000 / (1 + 83e^{-1.26t}) + ((\cos(6.28t)\, 55{,}000) / (1 + 150e^{-1.26r}))$.
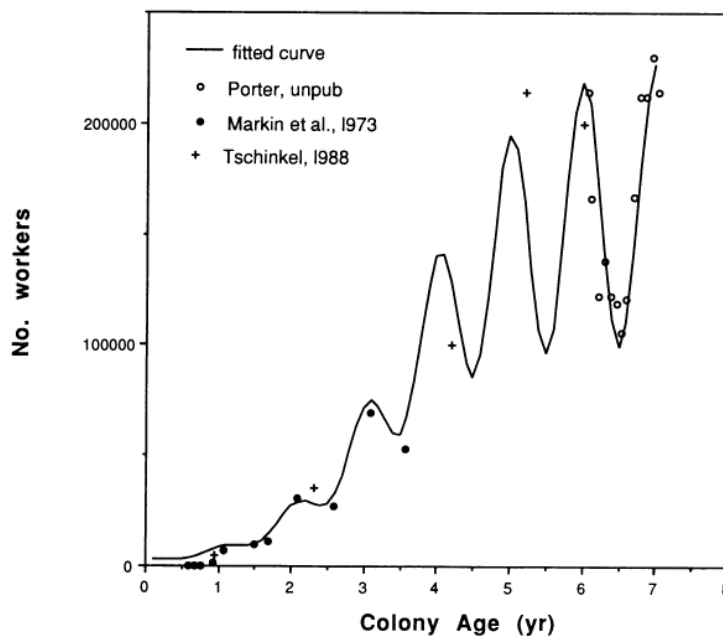

Figure 2: Curve fitted to the data of Tschinkel (1988), Markin *et al.* (1973) and Porter (unpublished). Adapted from (Tschinkel, 1993a).

Cassil et al (2002) provide colony growth curves from claustrally founded (monogyne) lab colonies. Environmental conditions within the lab are not stated. Growth was exponential after four months, reaching nearly 2,500 workers by 24 weeks (Figure 3).
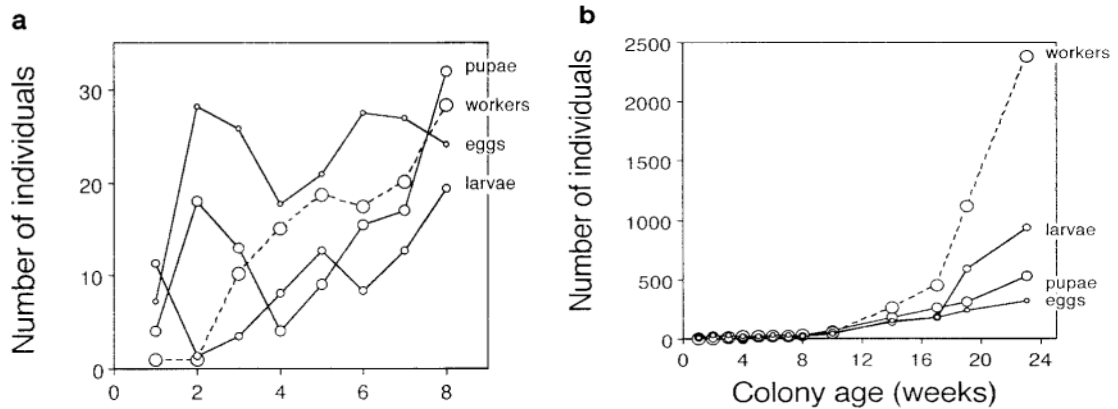

Figure 3: Number of workers, larvae, pupae and eggs produced by claustrally founded monogyne queens under lab conditions a) up to eight weeks old b) up to 24 weeks old. Adapted from Cassill (2002).

Colony sizes by age as reported in four different papers are summarised below in Table 1.

Table 1: Colony sizes reported in four papers at various colony ages. The locations of samples- laboratory or field, and environmental conditions experienced by laboratory-reared colonies are noted. Numbers are means unless otherwise stated.

Colony Age	(Tschinkel, 1988) Laboratory and field colonies, Florida. mean (S.D.)	(Cassill, 2002) Claustrally founded lab colonies Env. conditions not given, possibly 29°C	(Wood & Tschinkel, 1981) Laboratory Abundant food, 28°C	(Markin, Dillier, & Collins, 1973) Field colonies, Mississippi mean (range)
30 days				16 (3-24)
1 month			*c.* 100	
60 days				66 (16-85)

Age				
90 days				218 (58 – 1,340)
3 months			c. 1000	
4 months		onset of exponential growth		
5 months	1926 (560)			1,110 (650 – 2,400)
6 months	8076 (2120)	c. 2,400 workers		
7 months			8,000	6,576 (1,850 – 19,400)
1 year				11,073 (5,557 – 31,314)
1.5				29,989 (10,013 – 79,348)
2				26,888 (11,021 – 46,382)
2.5				69,162 (26,408 - 200,000)
2.5-3.5 years	Half sized colonies			
3 years	c. 100-150,000 workers but up to 230,000 have been recorded			52,741* (31,408 - 188,000)
4-6 years	Full sized colonies c. 220,000			

* Markin et al. (1973) also state in their text that their colonies reached an average of 120,000 workers. The 53,000 estimate is preferable as they repeat it in the text as well as their data table.

Markin et al. (1973) report that mature field colonies generally comprise 120,000 workers, but do not provide a source for this, nor a definition of 'mature'.

4.1.1 Methods of Determining Colony Size

Mound Volume

Field colonies in Florida showed a positive relationship between mound volume and colony population, with mound volume paralleling colony size annual fluctuations (Figure 4) (Tschinkel, 1993a). Total mass of ants explained 85% of the variation in mound volume. Seasonal effects on volume were significant, but relatively small. The model (LogV= -3.82 + 1.06logW) was less accurate for smaller colony sizes, where field data showed that the smallest mounds sampled contained 4500-9400 workers, whereas the graph intercept predicts 9,000 to 14,000 workers (Tschinkel, 1993a).
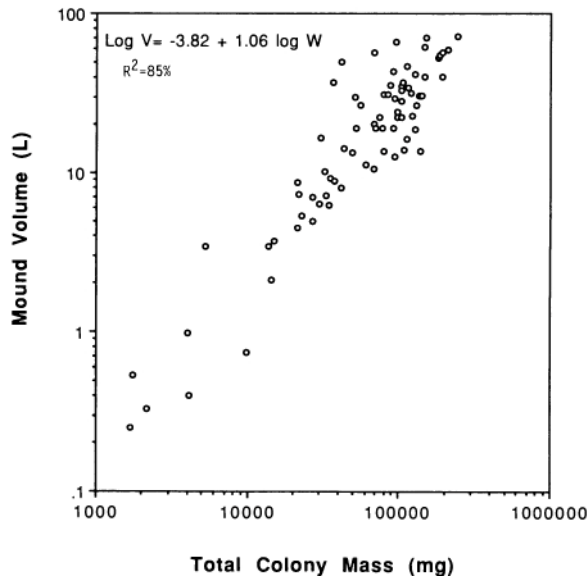

Figure 4: Mound volume in relation to colony mass. Data from field colonies in Florida. Linear model and r^2 are noted top right (Tschinkel, 1993a).

Markin et al. (1973) recorded mound diameter of field colonies in Mississippi (Table 2). Older colonies were found to move occasionally, abandoning the old mound and constructing a new one (Markin et al., 1973). This reduces the accuracy of mound size for estimating age of older colonies.

Table 2: Summary of mound diameters recorded by Markin et al. (1973), from field colonies in Mississippi. Sample sizes from Table 1 apply.

Age of Colony	Avg diameter of mound
< 90 days	5-8 cm
4 months	8-12 cm
5 months	12-18cm
7 months	18-25 cm

Worker Extraction

Worker abundance in small colonies may be counted by spreading the nest in shallow trays and collecting ants in a trap nest as the soil dries (Markin, 1968). Larger nests may be sampled using flooding techniques, whereby a sample of the nest is gradually flooded so ants float to the surface to be collected (Markin et al., 1973; Tschinkel, 1988; Tschinkel, 1993). Ants can then be counted to estimate colony size, or weighed and measured to estimate colony age (see below).

4.1.2 Confounding Effects-Colony Size:

Temperature

Soil temperature is generally considered the key ecological factor determining colony activity and metabolism (Killion & Grant, 1995; Korzukhin, Porter, Thompson, & Wiley, 2001). There is a positive linear relationship between minor workers' brood development time and temperature (55 days at 24°C to 23 days at 35°C) (Porter, 1988). Brood production in field colonies in Florida was 20% higher at warmer, sunny sites, compared to shaded sites, but total production and queen survival was higher at the shadier, cooler site. Proportional development time of each developmental stage was unaffected by temperature (Tschinkel, 1993a).

Temperature in above-ground mounds varies greatly throughout the day, while below ground temperatures are more stable (Tschinkel, 1993a). Workers caring for brood select optimum temperatures within the soil column for the caste and developmental stage of the brood (Porter, 1988; Porter & Tschinkel, 1993) and favour temperatures within the nest that are optimum for their caste (Porter & Tschinkel, 1993).

Life History

Mean colony size decreases with increasing proportion of polygyne nests in Louisiana. The authors attributed this to mean nest size of polygyne colonies being smaller (Milks, Fuxa, Richter, & Moser, 2007).

Number of minims produced in laboratory conditions is highest for monogyne queens (Gp-9^{BB}), followed by large polygyne queens (predominantly Gp-9^{Bb}) and then light polygyne queens (predominantly Gp9-^{bb}). Light polygyne queens still reared nearly 20 workers on their own (DeHeer, 2002). Pleometrotically founded colonies (ten queens) in lab conditions reared sexuals more than 100 days earlier than haplometrotically founded colonies. The authors concluded that this

was due to the pleometrotic colony attaining the size required for reproductive production earlier (Vargo, 1988).

Seasonal Fluctuations

Colony sizes vary seasonally (Tschinkel, 1988). Colonies reach maximum population size in mid-winter and reach a mid-summer minimum after spring sexual production. However, biomass maximises in spring due to high number of larger workers (Figure 5) (Tschinkel, 1993a).
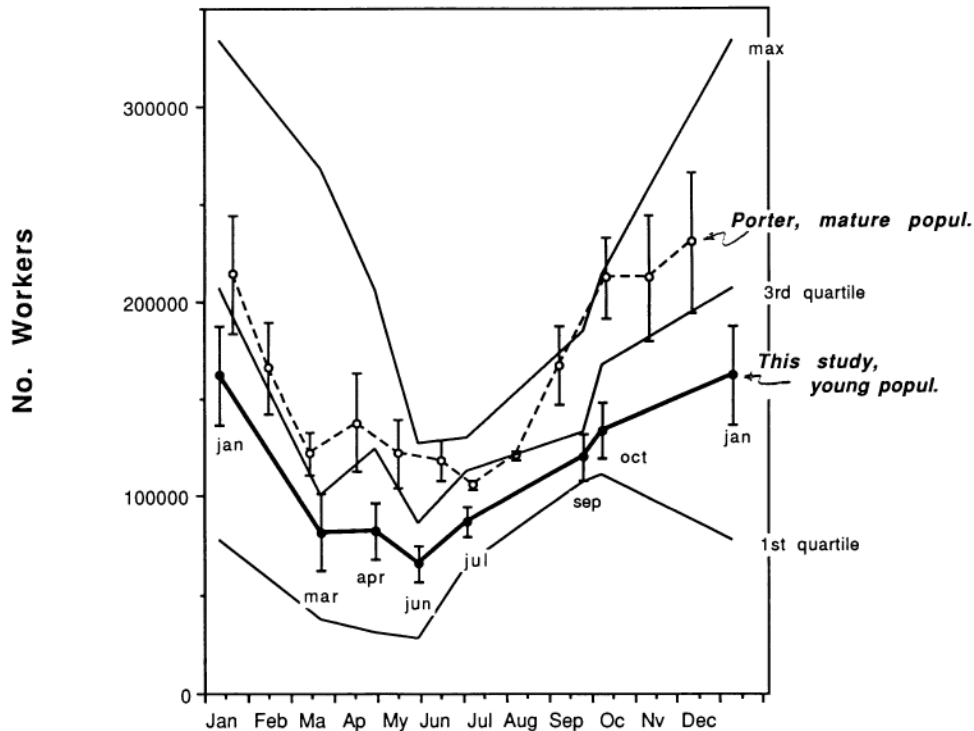

Figure 5: Mean worker population through an annual cycle from two studies in Talihassee, Florida. 'Porter' study is of a mature colony, 'this study' is of a younger colony. First quartile, third quartile and maximum colonies are shown for 'this study'. The mean seasonal variation in colony size was similar in both studies. Error bars = ±1s.e. (Tschinkel, 1993a).

Age Effects

The production and flight of alates by a colony reduces its size (Markin et al., 1973; Tschinkel, 1993a). This makes age determination more difficult in colonies that have produced alates, as the magnitude of this reduction is often uncertain (Tschinkel, 1988).

Food Availability

Preliminary field experiments in Florida indicated colony mounds were larger in higher food areas when compared to adjacent to lower food areas. In the high food areas, colonies were thought to reach maximum size for the environment within one year (Tschinkel, 1988). Well-fed laboratory colonies reached an order of magnitude more than food limited colonies (Figure 6) (Porter & Tschinkel, 1993).
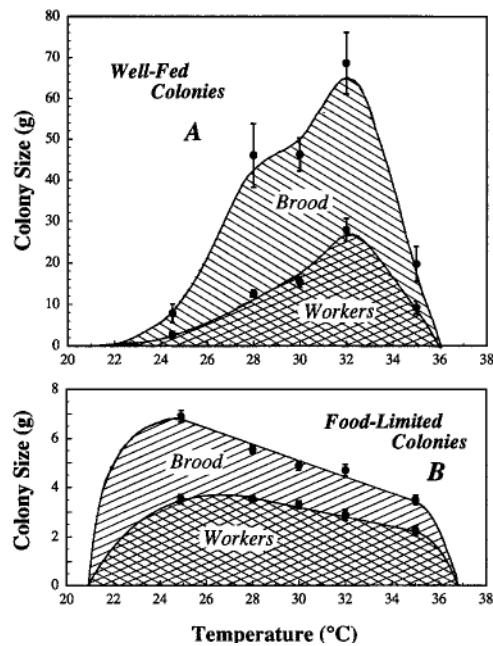

Figure 6: Effects of temperature on the size of **A** well fed colonies and **B** food limited colonies after two months. In each figure, total colony weight is separated in to mean worker and mean brood proportions. Lines are fitted by eye. Error bars in **A** are ±1SE. Adapted from (Porter & Tschinkel, 1993).

Laboratory colonies required 1.14 ± 0.29 Keal of food per gram of colony per week to sustain themselves (Macom & Porter, 1995). The type of food provided also affects colony growth, with sugar-water increasing colony growth by 60% in laboratory conditions (Porter, 1989)**.**

Food Availability and Temperature Interaction

Maximum growth in laboratory colonies occurs at 32 degrees in well-fed and at 25 degrees in food-limited colonies (Figure 6) (Porter & Tschinkel, 1993). The authors attributed this to lower metabolic costs to workers at lower temperatures.

4.2   **Worker Head Widths** (Tschinkel, 1988; Wood & Tschinkel, 1981)

When all worker castes are considered together, mean head width distribution changes with colony age due both to the production of new castes as the colony ages, and to changes in mean head width of a caste (Tschinkel, 1988; Wood & Tschinkel, 1981). Incipient field colonies demonstrated a mean head width of

0.51mm, because they consisted exclusively of minim/nantic workers. The modal head width increased for about 6 months, then remained between 0.55 and 0.75mm. From 1 month, the size frequency became skewed to the right, due to the addition of workers with head widths 0.75-1.6mm (Figure 7; Tschinkel, 1988).
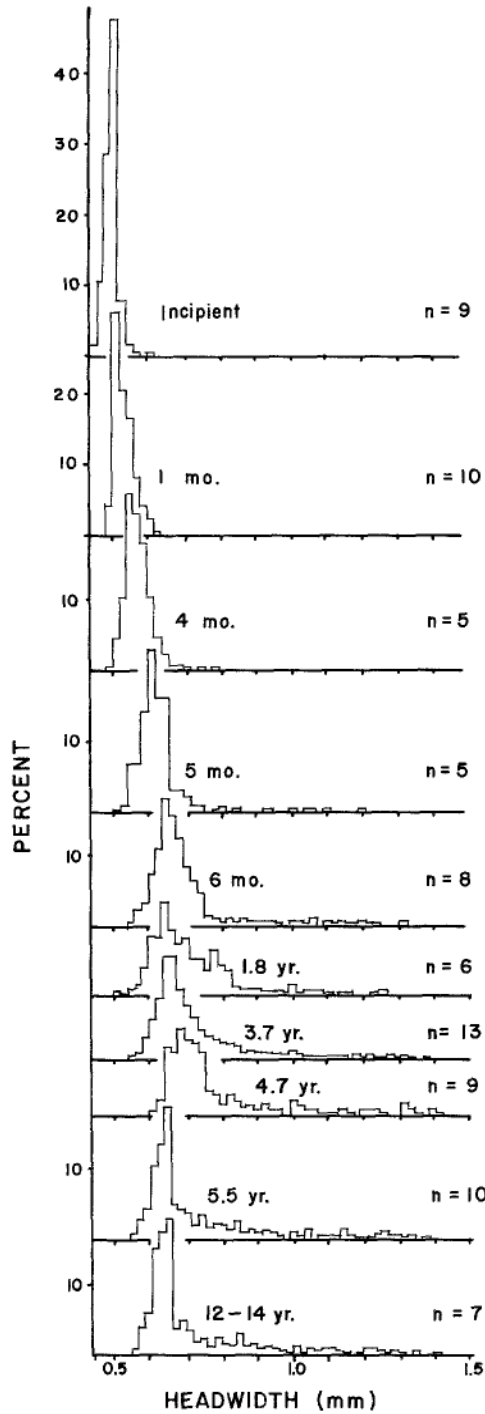

Figure 7: Headwidth-frequency distribution of workers in field colonies of increasing age from Florida. Each histogram is the sum over all colonies (*n*) of that age and is shown as percent of total. (Tschinkel, 1988).

Laboratory colonies, of unknown social form, provided with abundant food at 28°C demonstrated a similar pattern of head width growth, as summarised in Table 3.

Table 3: Summary of head width intervals present in *Solenopsis invicta* colonies at one, three and seven months (Wood & Tschinkel, 1981).

Colony Age	Mean Worker Head Widths
One month	Head widths between 0.45 and 0.7mm. Mode and mean similar in most colonies, or mode slightly lower. Onset of skewness after three months.
Three months	Head widths: 0.45-0.7mm. Modal class of 0.6-0.7mm established here and retained for remainder of the experiment.
7 months	0.5-1.5mm

4.3   Caste Proportions by Worker Weight (Markin et al., 1973)

Field colonies within Mississippi were dug up and workers counted by floating. Castes were arbitrarily defined and are shown in Table 4.

Table 4: *S. invicta* classes as defined by Markin et al (1973).

	minim	minor	major
Worker weight	<0.5 mg	0.5- 2.0 mg	>2.0 mg

Composition of colonies at each age are summarised in Table 5. Details of the calculation of these proportions were not provided.

Table 5: Proportional caste composition of *S. invicta* field colonies from Mississippi, as calculated by Markin et al. (1973).

Age	Sample size	% Minims	% Minors	% Majors
30 days	14	100	0	0
60 days	8	92	8	0
90 days	6	21	79	0
5 months	5	0	99	1
7 months	20	0	97.4	2.6
1 year	16	0	95.4	4.6
1.5 years	14	0	94.2	5.8
2 years	9	0	91.9	8.1
2.5 years	21	0	93.8	6.2
3 years	6	0	87.8	12.2

4.4 Caste Proportions by Worker Head Width

Laboratory colonies were provided with abundant food at 28∘C and monitored for up to seven months (Wood & Tschinkel, 1981). Social form of the queens (monogyne/ polygyne) was not specified. Queens were collected from Tallahassee, Florida.

Castes were arbitrarily defined by head class as summarized in Table 6.

Table 6: Caste size classes as defined by Wood and Tschinkel (1981).

	Minim	Minor	Media	Major
Head width (mm)	0.45	0.46-0.8	<1.1	<1.5

Minims emerged in all replicates by 36 days, comprising 100% of workers and were present until 3 months. By 29 weeks, minors, media and majors comprised 88%, 11% and 1%, respectively (Figure 8).
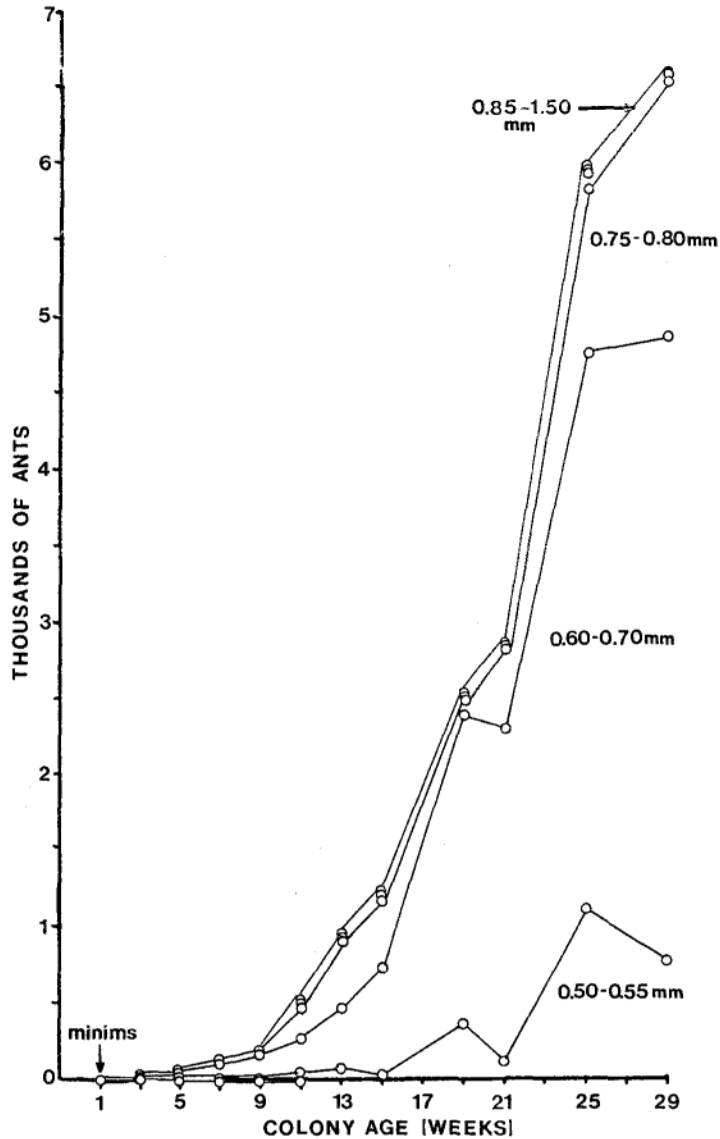

Figure 8: The average number of ants in laboratory colonies as a function of time. The mean number of ants in each size class is added to that of the previous size class. The interval between each curve indicates the number of ants in that size range. Adapted from Wood & Tschinkel (1981).

Normal scores as a function of head width distributions of RIFA field colonies older than four moths demonstrate a kinked-straight line pattern, indicating that colonies are comprised of two slightly overlapping, normally distributed populations (Figure 9) (Tschinkel, 1988).
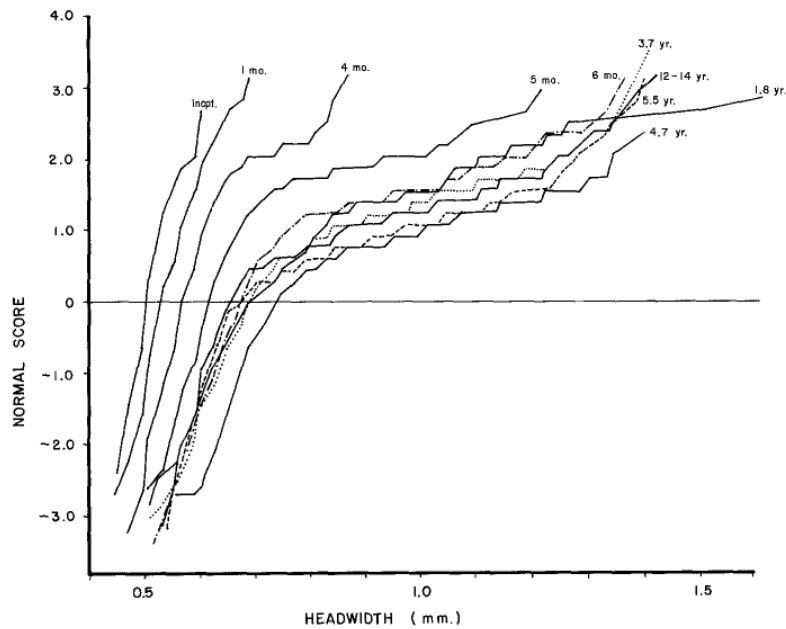
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Figure 9: Normal scores of colonies from ten age groups, as a function of head-width. All colonies sampled within an age group were combined (Tschinkel, 1988).

Tschinkel (1988) defined major and minor castes by which of these two distributions a worker belonged to. The mean head widths of both castes increased until *c.* 6 months, then remained around constant until 4.7 years (minors ANOVA F $1,60 = 36.6$; $p<0.001$; majors ANOVA : F $1,60 = 33.8$, $p<0.001$) (Figure 10). A decrease in minors' head width after 5.5 years was significant at the five percent level, but the decrease in majors' head widths was not significant.
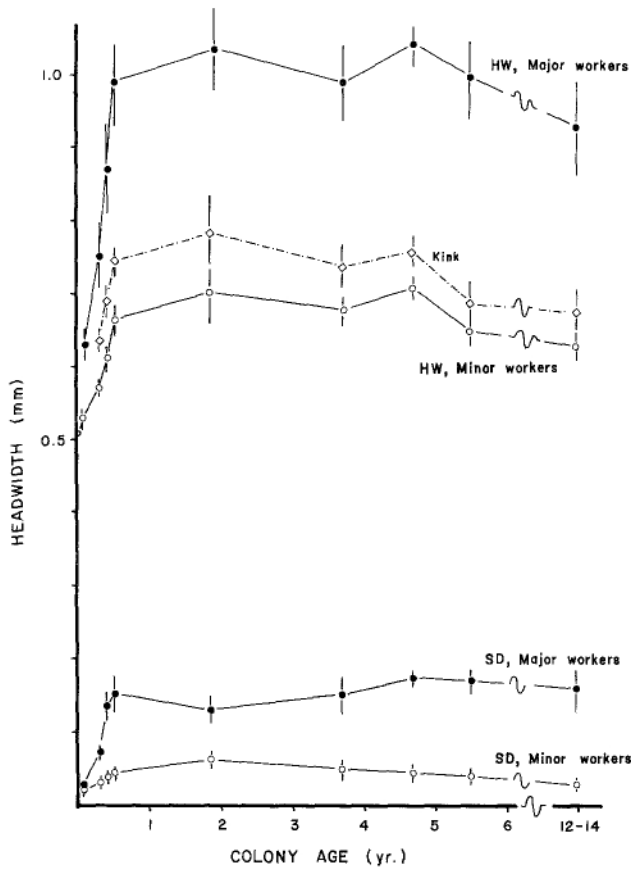
Figure 10: The increase in the mean size and size variability of major and minor workers in relation to colony age, based upon field colonies in Florida. HW= headwidth, SD= standard deviation of head widths (lower two graphs). Error bars= S.D. of the plotted means (Tschinkel, 1988).

Numbers of majors increased in a logistic function along with colony growth, but at a slightly faster rate, resulting in an increasing proportion of major workers as the colony aged. The proportion increased up to 5.5 years, then remained constant at around 35% (Figure 11).
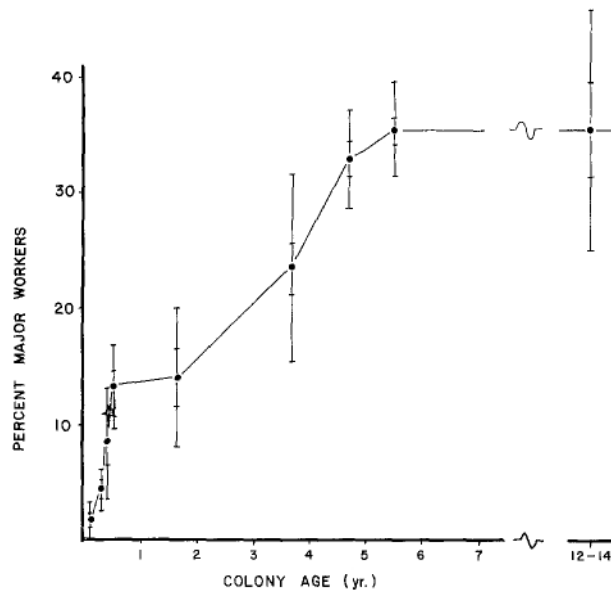
Figure 11: The proportion of total worker population that is major workers, as a function of colony age. Adapted from (Tschinkel, 1988).

Porter and Tschinkel (1985) divided RIFA into head width castes based upon their passage through a stack of sieves of decreasing mesh size (Figure 12). The proportion of each caste by abundance and mass are given in Figure 12. Data are totals from 34 field colonies in Florida that were judged to be two to three years old, based upon comparison with colony sizes reported by Markin et al. (1973).
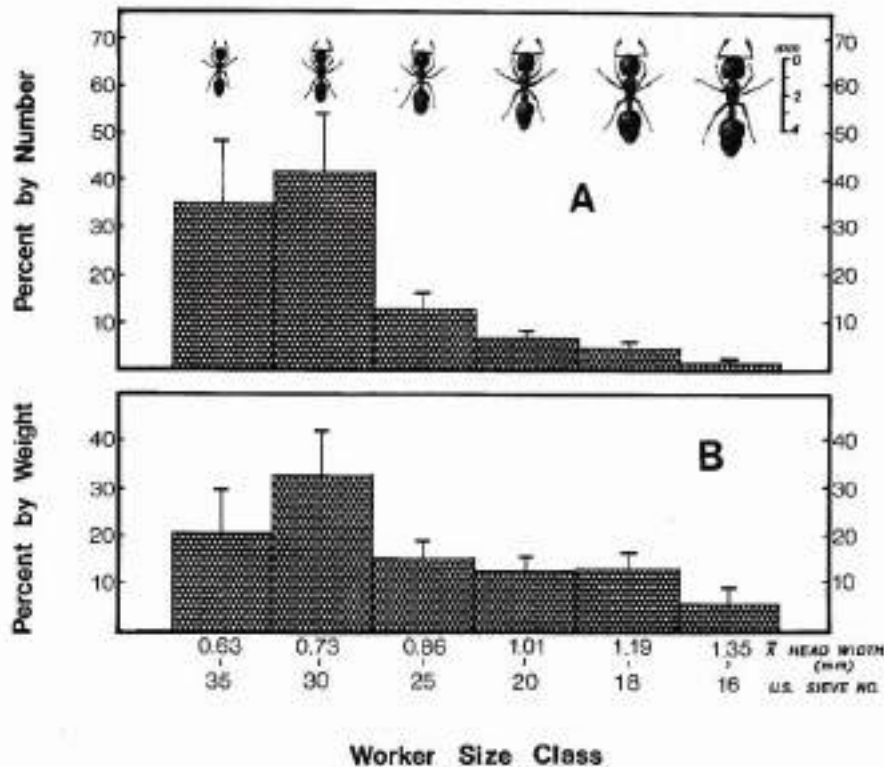
Figure 12: A: Percent distribution of worker population by size class. B: Percent distribution of worker biomass by size class. Data are averages from thirty-four fire ant colonies in Florida. Error bars are standard deviations.

Porter and Tschinkel (1985) also reported that worker head width in these 34 colonies was related linearly to body weight on a log-log plot (y= 0.933 $x^{2.98}$, r^2= 0.93).

In 2006, Porter recommended the caste head widths outlined in Table 7 to MAFBNZ for assessment of colony age[1].

Table 7: Head width classes of *S. invicta* worker castes, as recommended by Porter (2006).

	Majors	Mediums	Minors
Head width class	> 1.3 mm	0.76- 1.2 mm	0.6- 0.75 mm

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[1] Dr. Porter was not contactable regarding the basis for these recommendations, or their comparability to other authors' caste divisions.

4.4.1 Confounding Factors- Head Widths

Life History
In Louisiana, significant overlap of head widths between the social forms (monogyne and polygyne) was found in field colonies – monogyne workers in the top 90% of head widths had a 75% chance of misclassification (Colby, Inmon, & Foil, 2007).

Resource Availability
Food availability and competition for resources can theoretically affect worker size but this has not been tested experimentally (Colby et al., 2007).

Seasonal variation

Mean worker size fluctuates seasonally. Mean sizes peak in spring and are smallest in autumn (Tschinkel, 1993a).

4.5 Worker Fat Content

Percent fat content generally increased with colony size in field colonies (Tschinkel, 1993a), and therefore could be related to colony age. However, the difference was only significant between July and January (mid-summer to mid-winter), so seasonality would have to be considered when using this methodology. This is the only paper to have investigated the relationship thus far.

4.6 Models of Colony Growth

A colony-growth model has been developed by Killion and Grant (1995). It relies primarily upon temperature driven development rates to model the number of workers in a colony from foundation to maturity. It does not account for precipitation, soil moisture or food availability. The model's predictions deviated significantly from field data in the early period following establishment (60-120 days) but mapped later periods well (Killion & Grant, 1995).

Korzukhin et al. (2001) have developed a model of maximum geographic limits. It is a "dynamic model of growth using two time steps per day". Empirical data used include growth rate with temperature (Porter, 1988) and worker longevity with temperature (Calabi & Porter, 1989; Porter, 1988; Tschinkel, 1993).

4.7   Age at Production of Sexuals

Production of sexuals (alates) is linked to colony size (Vargo, 1988), but not to number of queens. Laboratory colonies (monogyne, abundant food, 29± 2°C, 14 h light/day) founded by single queens (haplometrosis) and ten queens (pleometrosis) showed a non-significant difference in colony size at production of alates (abundant food provided). Both treatments began to produce sexuals at 45-65 grams (about 22,500 to 32,500 workers; *c.* 500 workers/colony gram) (Vargo, 1988).

The earliest production of alates in field colonies in Mississippi was as colonies increased their average number of workers from 1,110 at 5 months to 6,576 at 7 months (Markin et al., 1973). Only two out of twenty colonies contained alates at this point. The number of colonies with alates peaked in 21 colonies (100%) at 2.5 years old (average= 69,162 workers) (Markin et al., 1973). Frequency of alate production is summarised in Table 8.

Table 8: Percent of field colonies in Mississippi containing alates, compared with colony age. Sample size is noted. Adapted from (Markin et al., 1973).

Colony Age	Sample Size	Average Colony Size	Percent of colonies with alates
30 days	14	16	0
60 days	8	66	0
90 days	6	218	0
5 months	5	1110	0
7 months	20	6576	2
1 year	16	11,073	12
1.5 years	14	29,989	6
2 years	9	26,888	9
2.5 years	21	26,408	21
3 years	6	31,408	6

4.7.1 Influences on Sexual Production

Possible influences on the production of alates include queen influences, worker:larvae ratios and food supply (Vargo, 1988).

Queens emit a pheromone that inhibits alate production by modifying worker behaviour. Inhibition strength is proportional to worker: queen numbers: more queens can inhibit larger numbers of workers, but at low queen numbers, inhibition is low and alates may be produced (Vargo & Fletcher, 1986a, 1986b)

5. Conclusions

Empirical data regarding methods of ageing RIFA colonies is limited. Many of the earlier papers lack crucial information such as social form of test subjects, environmental conditions within the laboratory, or error measurements. Field data comes only from the USA. Laboratory studies are limited to claustrally founded colonies.

Five methods of ageing RIFA colonies have been found: colony size, mean worker head width, caste proportions by mean head width, caste proportions by worker weight and worker fat content. Worker fat content currently lacks the empirical data to be considered a useful methodology. Further research would be required its assessment as an ageing technique. The remaining methods all have confounding influences that are poorly understood and, where they are reported in more than one paper, demonstrate variability between studies. Therefore, assessment that integrates all four methods would provide the most reliable estimate of colony age.

Colony Size

Both empirical and computer models of colony size are least accurate at small colony sizes. This may not be a practical issue as incursions are likely to be noticed only once they reach large numbers. Temperature is thought to be the most important factor affecting colony growth, but its effects are poorly understood at larger time scales. Microclimates within establishment areas must specifically be considered. Collecting temperature data from sites of future incursions would provide information regarding the levels of temperature variation experienced by the colony and the types of microclimates favouring colony establishment.

Food availability also affects colony growth, but has been little studied. A colony invading a new area, such as in an incursion, may experience unlimited food resources. However, if the habitat is inhospitable, such as at a port (a tarmac surface), food resources may be limited.

The use of mound size as a surrogate for colony size should be used cautiously due to confounding factors. In addition, incursions occurring at ports and airports will often be underneath a tarmac surface which will affect mound creation.

Caste Proportions

The variability between authors' definitions of RIFA castes makes comparisons between studies difficult. Assessment of the comparability of head width- defined castes (Tschinkel (1988), Wood & Tschinkel (1981)) with Markin et al's (1973) weight- defined castes would be particularly useful. This should include investigations in to the applicability of the head width-weight relationship (Porter & Tschinkel, 1985) to colonies in other areas and the effects of seasonal variation on this. Sanford Porter and Walter Tschinkel may be able to provide information here.

Only Tschinkel's (1988) head width- defined castes are based upon empirical examination of RIFA field colonies. However, this method does not provide finite categories for worker castes, as mean head widths varied with age. Combinations of this methodology with colony size estimates (e.g. worker abundance) would be necessary to interpret data from nests of unknown age.

Alate Production

Laboratory data suggests colony size is crucial for the onset of reproduction, but there is disagreement between laboratory and field experiments regarding the size at which colonies produce alates. Under certain conditions, colonies can produce alates while still very small. Factors influencing alate production are an essential piece of information. Data has only been obtained on monogyne colonies.

5. Recommendations

- Further assessment should be made of the comparability of caste classifications present in the literature.

- Where possible, floating techniques should be used for the estimation of colony size.

- Tschinkel's (1988) definitions of worker castes should be favoured for head width based caste proportions.

- Research efforts should focus on factors that confound colony estimates, with particular reference to New Zealand conditions.

- Soil temperature loggers should be placed at varying soil depths at sites of future incursions and left in place for at least a year.

- Influences from environmental conditions should be considered in laboratory experiments.

- Research should be conducted into factors affecting alate production, with particular reference to colony size and polygyne colonies.

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7. References

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