Analysis of Ruwan's contraction data
by Osborne, March 27, 2006

The contraction data arise from a time-course experiment in which constructs serve as experimental units. Three constructs are randomized to each combination of three factors: medium, density and strain, in a complete, crossed 2×2×3 factorial design. One construct was lost for the (medium=B, density=30, strain=12) construct, resulting in 35 time profiles. Contraction is measured for each construct at each of 15 time points, ranging from time=0 to time=14. The total number of measurements available for analysis is then $35 \times 15 = 525$.

Two analyses might be considered. One approach is to compare the time profiles for the 12 treatment combinations. Construct effects and intra-construct correlation may be accounted for in a mixed model with fixed effects for medium, density, strain and *time* and random effects for construct. The $F-$test for equal profiles from this model is highly significant on $F = 7.67, p < 0.0001, df = 154, 322$. Subsequent F-tests comparing profiles indicate many effects of the three factors on the contraction profiles in time that are not easily quantified. One approach is to approximate the time profiles using some parameterization of the time effect. A second order polynomial provides a reasonable fit. The way the contraction measurements decrease in time may be investigated by inspection of the way the first and second-order coefficients vary according to the 12 treatment combinations. The time 0 measurements cannot be accomodated by the quadratic model (see the violation indicated by Figure 1). Since there is little variability in contraction measurement at this time point, the quadratic model may be fit using only the subsequent measurements. The model is then

$$Y_{ijkl}(t_m) = \mu + \alpha\beta\gamma_{0ijk} + (\alpha\beta\gamma)_{1ijk}t_m + (\alpha\beta\gamma)_{2ijk}t_m^2 + C_{l(ijk)} + \epsilon_{ijklm}$$

where ijk indexes the factorial treatment combination, l indexes construct and m indexes time. C and ϵ are random construct and error effects respectively. (A more complete exposition of the model, in which the $(\alpha\beta\gamma)_{0...}$ $(\alpha\beta\gamma)_{1...}$ and $(\alpha\beta\gamma)_{2...}$ terms are broken into their main effect and interaction components will not fit on this page.) The F-ratios and p-values for tests of equal coefficients for the *time* and $time^2$ terms in the quadratic model are listed in the output on the next page. Additionally, the estimated variance components for the random effects are given and indicate substantial within-construct correlation.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Cons(Dens*Medi*Stra)	7.8033
Residual	13.8699

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Density	1	23	78.88	<.0001
Medium	1	23	26.98	<.0001
Density*Medium	1	23	5.13	0.0332
Strain	2	23	0.42	0.6613
Density*Strain	2	23	29.17	<.0001
Medium*Strain	2	23	50.41	<.0001
Densit*Medium*Strain	2	23	12.18	0.0002
Time	1	431	1026.53	<.0001
Time*Density	1	431	25.28	<.0001
Time*Medium	1	431	2.47	0.1170
Time*Density*Medium	1	431	7.43	0.0067
Time*Strain	2	431	2.71	0.0677
Time*Density*Strain	2	431	15.22	<.0001
Time*Medium*Strain	2	431	5.07	0.0067
Time*Dens*Medi*Strai	2	431	10.12	<.0001
Time*Time*Strain	2	431	0.49	0.6132
Time*Time*Dens*Strai	3	431	9.05	<.0001
Time*Time*Medi*Strai	3	431	1.71	0.1640
Tim*Tim*Den*Med*Stra	3	431	5.35	0.0013
Time*Time	1	431	254.98	<.0001

A plot of fitted values from this model is given in Figure 2. It is not particularly useful for elucidating factorial effects on contraction. The F-tests indicate that they do have an effect on the way contractions decrease over time, with some of the linear and quadratic coefficients depending on density, medium and strain combinations.

Another approach to analyzing the effects of the experimental factors on contraction is to boil the time profile down to a single quantity, such as the area under the curve (AUC). Those profiles with a more rapid decline in contraction over time will have lower AUC values. Further, the interactions of the factorial effects on contraction over time can be quantified through a single change in AUC, as opposed to differences in linear and quadratic coefficients as in the quadratic approximation model. Lastly, there is no serial correlation to worry about, as there is but a single AUC response for each of the 35 constructs.

The statistical model for this approach, which is considerably simpler to fit and interpret than the longitudinal and quadratic models considered above is given by

$$AUC_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl}$$

where i, j, k and l are defined as before. The ANOVA table for the analysis of these AUC measurements are given below and the interaction plot appears as Figure 3:

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                          analysis of AUC msmts                          6
                                              14:04 Monday, March 27, 2006

                            The GLM Procedure

                                     Sum of
Source                     DF       Squares   Mean Square  F Value  Pr > F
Model                      11   396141.0824    36012.8257    20.93  <.0001
Error                      23    39569.0998     1720.3956
Corrected Total            34   435710.1822

           R-Square     Coeff Var      Root MSE      auc Mean
           0.909185      7.059017      41.47765      587.5840

Source                     DF   Type III SS   Mean Square  F Value  Pr > F
Medium                      1   138299.0689   138299.0689    80.39  <.0001
Density                     1    46882.1845    46882.1845    27.25  <.0001
Medium*Density              1      204.3568      204.3568     0.12  0.7335
Strain                      2    26146.9102    13073.4551     7.60  0.0029
Medium*Strain               2   177915.8007    88957.9003    51.71  <.0001
Density*Strain              2    18164.5401     9082.2700     5.28  0.0130
Medium*Densit*Strain        2     1750.8657      875.4329     0.51  0.6078

                         Least Squares Means

                Medium    Density    Strain      auc LSMEAN
                B         30         0           682.048873
                B         30         10          609.350275
                B         30         12          780.396500
                B         60         0           593.928264
                B         60         10          478.971216
                B         60         12          792.498488
                O         30         0           573.599714
                O         30         10          641.221976
                O         30         12          492.009695
                O         60         0           470.226340
                O         60         10          538.565413
                O         60         12          462.461707
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The complex effects of the three factors on contraction may be summarized with the following observations:

1. There is a clear strain-by-medium interaction. For medium B, the strain effect is nonlinear, with a minimum contraction AUC at strain=10 with higher AUCs at strain=0 and strain=12. For medium O, the strain effects is still nonlinear, but with the opposite direction, with a maximum contraction AUC at strain=10 with lower AUCs at strain=0 and strain=12. The F-ratio for this first-order interaction is highly significant $F = 51.7, p < 0.0001, df = 2, 23$ and this term explains more variance than in AUC than any other term in the model ($SS(medium * strain) = 177916$).

2. There is a significant strain-by-density interaction. The density effect is very constant for strain < 10, with higher AUC for 30 than 60, but appears to be very diminished at strain=12. The strain-by-density effect is significant, ($F = 5.28, p = .01, df = 2, 23$) but not nearly as pronounced as the strain-by-medium interaction described in 1.

3. These two interaction are fairly consistent across levels of the other factor. The strain-by-medium interaction is quite consistent across densities, or the strain-by-density interaction is consistent across the mediums. The second-order interaction of strain, density and medium is not at all significant ($p = 0.6078$).

To further explore these observations, some individual pairwise comparisons may be made. For the strain-by-medium interaction, the two mediums may be compared separately after averaging over density. These comparisons are the first three items listed in the output below. For the strain-by-density interaction, the two densities may be compared separately after averaging over medium. These appear as the last three items listed in the output below. The standard errors do not quite agree due to the slight imbalance resulting from the one lost construct.

Parameter	Estimate	Standard Error	t Value	Pr > |t|
medium effect at strain=0	-116.075541	23.9471337	-4.85	<.0001
medium effect at strain=10	45.732950	23.9471337	1.91	0.0687
medium effect at strain=12	-309.211792	25.3997710	-12.17	<.0001
density effect at strain=0	-95.746992	23.9471337	-4.00	0.0006
density effect at strain=10	-116.517811	23.9471337	-4.87	<.0001
density effect at strain=12	-8.723000	25.3997710	-0.34	0.7344

Figure 1: Residuals against fitted values for quadratic mixed model, symbol=time

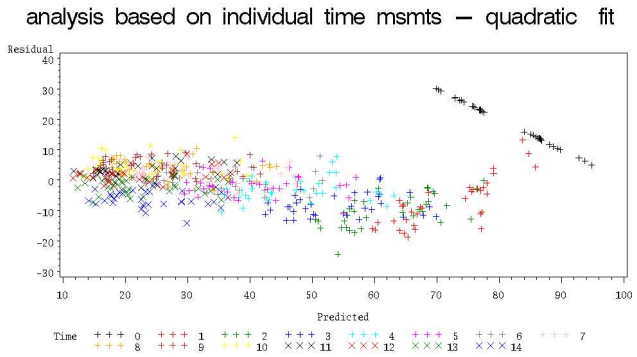

Figure 2: Quadratic fits for time profiles

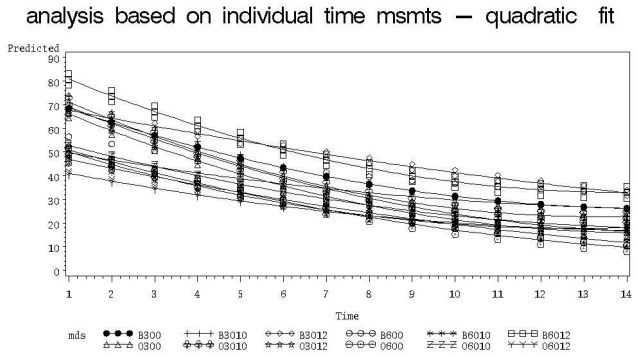

Figure 3: Interaction plot - AUC plot against strain for the four medium by density combinations